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The Effects of Cold Plasma Treatment on Sweet Basil (Ocimum basilicum)

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The Effects of Cold Plasma Treatment on Sweet Basil (*Ocimum basilicum)*

By

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THESIS

Submitted to the Department of Chemistry and Biochemistry at Seton Hall University in partial fulfilment of the requirements for the degree of Master of Science

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We certify that we have read this thesis and that in our opinion it is adequate to scientific scope and quality as a thesis for the degree of Master of Science.

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Table of Contents

List of Figures

List of Tables

Abstract

The study discussed herein looks both to continue and to expand upon previous work conducted here at Seton Hall University, which investigated the effect of cold plasma processing on sweet basil and their essential oils. It was found that the application of cold plasma treatment increases plant growth and eugenol concentration, which is an essential oil component and one of the potential natural antioxidants used in food preservation. In this study, we considered methods for increasing the production and harvesting of plants that produce essential oils such as sweet basil (*Ocimum basilicum*). The study also incorporated more controls than the previous study which served as a pilot of the present study. These controls included: more sample groups, i.e., seed treated, plant body treatment once and twice a week; different parts of the basil plant were separately treated, such as the leaves, stems, and flowers; and a standard distance of plasma application was maintained. The main focus of the current study was to investigate in detail the effect of the cold plasma treatment on the overall growth of the sweet basil plants, to optimize the extraction protocol of the essential oils present, and to determine quantitatively the effect which the plasma treatment plays on the distribution of the main compounds found in the control and plasma treated plant groups. Sweet basil contains different components of essential oil such as eucalyptol, linalool, estragole, eugenol, and methyl cinnamate. Results of this study showed an increase of the essential oils extracted from the plant based on the plasma treatment. Also, results showed an increase in the essential oil extracted from the basil plant according to the intensity of the treatment either once a week or twice a week with 30 second per treatment. Estragole was the predominant component in the leaves and linalool was predominant in the flowers, both at the twice a week treatment.

Chapter 1

Introduction

Second only to the increase in agricultural production, the preservation and increased shelf life of food products is paramount to meeting the growing global demand. One common type of food preservation technique is to add antioxidants, which help to prevent or retard lipid oxidation. Lipid oxidation is a deteriorative process that causes oil or fat to turn rancid and food to spoil. Two synthetic antioxidants commonly used by the food industry are butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT). Although BHA and BHT are effective antioxidants, animal studies have shown that they are potentially harmful to human health, as reviewed by Amorati [1]. BHA has been shown to promote the action of some carcinogens, and BHT potentially causes lung damage [2,3,4]. These substances, however, have potential safety hazards. Food scientists have been seeking alternative natural compounds as substitute antioxidants. Numerous studies have shown such bioactive effects [5,6,7] with components originating in the plant's essential oils.

Among these natural alternatives are certain groups of chemicals, which are found in various plant species, called essential oils. Essential oils are aromatic oily liquids that are extracted from different parts of a plant and have been shown to demonstrate antibacterial and antioxidant potential for food preservation products due to their phenolic and polyphenolic constitution [8]. This effect can be measured by its scavenging activity of the free radical, DPPH (2-2-diphenyl-1-picrylhydrazyl), the Folin-Ciocalteau method, (reducing power assay) and β-carotene method [9]. Previous studies have also reported that various mixtures of naturally occurring essential oils act as active oxygen scavengers [10] and that these natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases [11]. Because of these benefits, studying natural antioxidants has grown increasingly more common and important. Different

assays have been used to quantify the antioxidant activity. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical quenching assay is considered one of the most authentic assays for antioxidant study [12, 17]. DPPH is an organic stable free radical which gives a purple color in solution and can be detected with maximum absorption at 518 nm (λmax) by UV/Vis spectroscopy [13,14].

Sweet basil is one of the most common food preparation herbs used in the world. *Ocimum basilicum* was used in our study for different reasons: economically (i.e., inexpensive) and relatively quick to grow. Basil contains natural constituents that can be of benefit for human health, including eugenol, estragole, linalool, and citronellol. These compounds are found in the 'sweet basil oil' and are defined as enzyme-inhibiting oils that can help lower inflammation, which is at the root of most diseases such as heart disease, rheumatoid arthritis and other chronic diseases. Understanding the potential chemical components of sweet basil, prior research with basil indicated that eugenol and estragole were the main chemical compounds; they are found to have potent antioxidant, antiviral, and antimicrobial properties [15].

Compared to the synthetic antioxidants (BHA, BHT), sweet basil contains two natural components (estragole and eugenol) that are suitable for food preservation. Eugenol was found to be the predominant component found in the extracted basil oil between the plasma treated and non-treated (control) plant using extraction by steam distillation [16] and GC-MS to identify the eugenol.

The word plasma comes from the ancient Greek, which means "moldable substance" or "jelly". It is describes the behavior of ionized atomic nuclei and electrons where each of the nuclei are suspended in a movable environment of electrons. Plasma was first identified in a Crookes tube in 1879 by Sir William Crookes where he called it "radiant matter" [17].

The term plasma was first discovered in 1928 by Irving Langmuir and it is considered as the fourth state of matter due to its structure substantially different from the three other states of matter such as solid, liquid and gaseous [18,19]. In terms of physics, plasma is used for sterilization purposes and composed of non-charged particles, molecules and radicals.

Plasma is formed when gases are left on constant current or between two electrodes where electrons and ions are unrestricted during plasma generation. This phenomenon affects cell walls of microorganisms. Studies show that plasma produces positive results in microbiological terms in various foods, such as vegetables, fruit and meat products [20,21]. There are two types of plasmas, cold and hot. The temperature of the ions of cold plasma is close to room temperature and thus has application in food [22].

The prior study which utilized atmospheric cold plasma jet (ACPJ) technology, has shown plasma treatment to be associated with an enhanced growth rate, an increase in the robustness of the plants, and perhaps most significantly, an enhanced antioxidant effect [16]. Even though the beneficial effects on the plant from the ACPJ treatment were noted, the mechanism causing the plant's growth and response is not yet understood. It is known that high-energy photons, excited free electrons, ions and neutral gas particle species are all being generated within the plasma jet effluent but the subsequent effect of each of these components on impact with the plant is yet to be determined. Additionally, even beyond the physical collisions of these plasma constituents, there is also the known reactive gas species such as N^+, H_2O_2, OH^-, H^+, NO, singlet oxygen, O_2, O_3, N_2O, and a few others like CO_2 and CO, that are formed and these activated chemicals are also suspected to play a role in the plant's response [23, 24]. The goal of this research is to understand the effect of cold plasma treatment on sweet basil by using qualitative and quantitative analytical techniques to analyze the essential oil extracted from sweet basil plants.

The current research is based on further study on the effects of cold plasma treatment on sweet basil, including plasma's potential effect on the chemical components of essential oil such as estragole, linalool, ocimene, eugenol, etc. The list of the chemical components, potentially present in sweet basil oil is shown in Table 1 and their chemical structures compiled in Figure 1.

Most of the components listed in Table 1 and Figure 1 belong to the terpene group which are part of the largest class of natural products. These components are found in plants such as star anise (*Illicium verum*), coriander (*Coriandrum sativum*), mint (mentha), and sweet basil (*Ocimum basilicum*). Sweet basil is one of the plants of interest consumed by humans for its various biological functions.

Table 1. Components Typically Found in Sweet Basil (*Ocimum basilicum*)

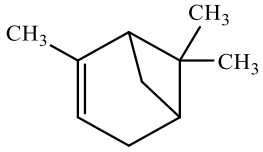
	Components	Formula	MW (g/mole)	Density (g/ml)
1	α-Pinene	$C_{10}H_{16}$	136.24	0.858
2	Myrcene	$C_{10}H_{16}$	136.24	0.791
3	β-Pinene	$C_{10}H_{16}$	136.24	0.859
4	*cis*-Ocimene	$C_{10}H_{16}$	136.24	0.818
5	Limonene	$C_{10}H_{16}$	136.24	0.841
6	*trans*-Ocimene	$C_{10}H_{16}$	136.24	0.818
7	Eucalyptol	$C_{10}H_{18}O$	154.25	0.922
8	Linalool	$C_{10}H_{18}O$	154.25	0.87
9	Camphor	$C_{10}H_{16}O$	152.23	0.99
10	Estragole	$C_{10}H_{12}O$	148.2	0.965
11	Terpineol	$C_{10}H_{18}O$	154.25	0.934
12	Citronellol	$C_{10}H_{20}O$	156.27	0.855
13	Geraniol	$C_{10}H_{18}O$	154.25	0.879
14	Eugenol	$C_{10}H_{12}O_2$	164.2	1.067
15	Methyl Cinnamate	$C_{10}H_{10}O_2$	162.185	1.09
17	Ethyl Cinnamate	$C_{11}H_{12}O_2$	176. 21	1.05

Source: Valgimigli, L; *Essential Oils as Natural Food Additives*, New York, Nova Science Publishers, **2012**.

Note: This particular sequence components are based on the retention time of the standard GC analysis.

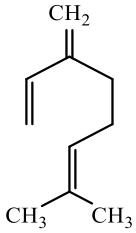
Figure 1. Chemical Structure of the Compounds in Sweet Basil from Table 1

1)



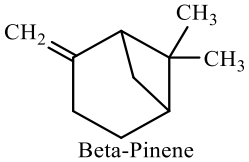
Alpha-Pinene

2)



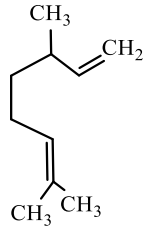
Myrcene

3)



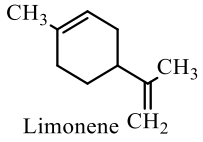
Beta-Pinene

4)



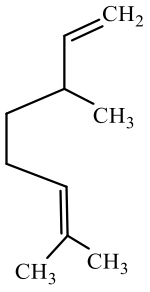
Cis-Ocimene

5)



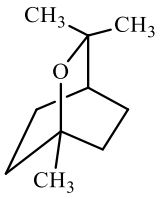
Limonene

6)



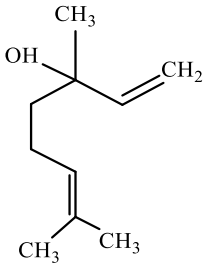
Trans Ocimene

7)



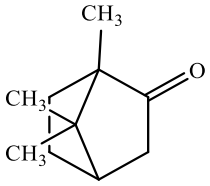
Eucalyptol

8)



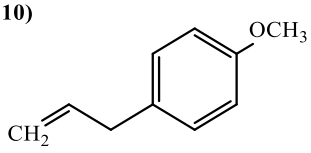
Linalool

9)



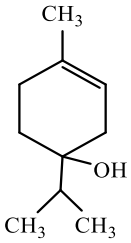
Camphor

10)



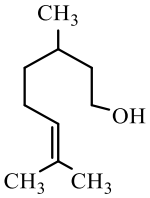
Estragole

11)



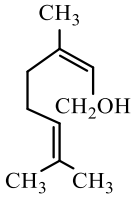
Terpineol

12)



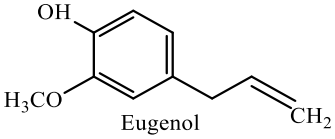
Citronellol

13)



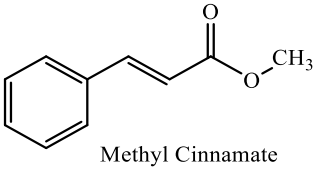
Geraniol

14)



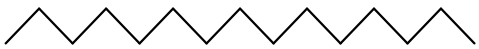
Eugenol

15)



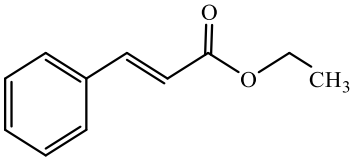
Methyl Cinnamate
or
Cinnamic Acid Methyl Ester (CAME)

16)



Penta Decane

17)



Ethyl Cinnamate
or
Cinnamic Acid Ethyl Ester (CAEE)

1.1. Health Benefits of Sweet Basil (*Ocimum basilicum*)

Basil leaves hold many significant plant-derived chemical compounds that are known to have disease preventing and health promoting properties [25]. Basil herb contains many polyphenolic flavonoids like orientin (flavone contains glucoside) and vicenin (a particular class of flavonoids that carry some protection against radiation damage). Previous studies show that the above polyphenolic components were utilized in *in-vitro* studies for their possible antioxidative protection against radiation-induced lipid peroxidation in mouse liver [26].

Basil leaves contain also many health-benefiting components of essential oils, such as eucalyptol, estragole, linalool, eugenol, citronellol, limonene, and terpineol. These components are known to have anti-inflammatory and antibacterial properties [27].

The herb is low in calories and contains no cholesterol. However, it is one of the finest sources of many essential nutrients, minerals, and vitamins. Basil contains exceptionally high levels of beta-carotene, vitamin-A, kryptoxanthin, lutein, and zeaxanthin. These components act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that can harm human health [28].

Based on the literature, basil contains zeaxanthin, a yellow flavonoid carotenoid, it is selectively absorbed into the retinal macula lutea where it is found to filter harmful UV rays from reaching the retina. Studies suggest that common herbs, fruits, and vegetables that are rich in zeaxanthin antioxidant help to protect from age-related macular disease (AMRD), especially in older adults [29,30].

One hundred grams of fresh herb basil leaves contain astonishingly 5275 mg or 175% of daily-required dose of vitamin A. Vitamin-A is known to have antioxidant properties and is essential for vision. It is also required for maintaining healthy skin in the human body. Consumption of natural foods rich in vitamin-A has been found to help protect their humans from lung and oral cavity cancers. In addition, Vitamin K in basil is essential for the production of clotting factors in the blood and plays a vital role in the bone strengthening and mineralization [31].

Besides other plants that contain different minerals used for human health, basil contains a good amount of minerals such as, potassium, manganese, copper, and magnesium. Potassium is an important component of cell and body fluids, which helps control heart rate and blood pressure. Manganese is used in the human body as an important co-factor for the antioxidant enzyme, superoxide dismutase [32, 33].

Basil leaves are an excellent source of iron. Its fresh leaves carry approximately 0.0317 mg/g (about 26% of RDA) of iron. Iron is a component of hemoglobin inside the red blood cells and it is one of the principal determinants of oxygen-carrying capacity of the blood [34].

1.2. Gas Chromatography / Mass Spectrometry (GC/MS) of Essential Oils

Different analytical methods have been used to analyze essential oils [35]. In 1952, modern gas-chromatography was invented by Martin and James [36], since then it has become one of the most important and widely applied analytical techniques in modern analytical chemistry. The beginning of fused quartz bonded-phase capillary columns has led to very reproducible retention times and reproducible columns from consignment to consignment [37], the succeeding coupling with an "ion trap detector" (ITD) [Finnegan Corp., 1987] and introduction of computer searching algorithms allowed unmistakable identification of essential oils (EOs) components based on retention times, the interpretation of mass spectra and spectral library search using dedicated software. Different types of detectors are coupled to capillary gas chromatography in order to analyze essential oils, including "flame ionization detector" (FID), "electron capture detector" (ECD), "thermal conductivity detector" (TCD). However the preceding detectors do not offer analyte identification as compared to detectors that are specific for certain classes of compounds: nitrogen-phosphorous detector (NPD), "flame photometric detector" (FPD), and "photo-ionization detector" (PID). These types of detectors do not provide structural information about the analytes [38].

Coupling of gas chromatography with mass spectrometry for separation yields better recovery of the essential oils with an online spectroscopic detector technology. These techniques include gas chromatography-mass spectrometry (GC-MS), GC- Fourier transform infrared spectroscopy (GC-FTIR), GC-nuclear magnetic resonance of carbon 13 (GC-CNMR) [39, 40]. The above techniques can obtain the structural information on the separated of essential oils components. GC-olfactometry (GC-O) is one of the separation techniques mostly used in the industrial field of the flavor and fragrances [41], which provides the characteristic configuration scent for essential oils combinations. Over the last decades, the analytical methods have been remarkably improved with broadened application in the analysis of essential oils (EOs). Different studies show that currently GC–MS equipped with chiral column is one of the most complete instrumentations, able to furnish specific data for quality control of essential oils.

Presently, the identification of essential oil components is usually carried out with the benefit of gas chromatography/mass spectrometry (GC/MS) equipped with flame ionization detector (FID) and mass selective detectors, and a capillary column which was used in the present study.

Chapter 2

Experimental

The main focus of this work was to investigate the effect of the cold plasma treatment on the sweet basil plants by determining the changes taking place in the component profile of the isolated essential oil possibly resulting from this treatment, to identify specific components, and determine whether they have been enhanced or diminished by plasma.

2.1. Materials

The following chemicals α-Pinene, Myrcene, β-Pinene, *cis*-Ocimene, Limonene, *trans*-Ocimene, Eucalyptol, Camphor, Linalool, Estragole, Terpineol, Citronellol, Geraniol, Eugenol, Methyl Cinnamate, and Ethyl Cinnamate were purchased from Sigma-Aldrich Co (Saint-Louis, MO). Hexane was purchased from Macron Fine Chemicals™ (Center Valley, PA USA). All of the chemicals and solvents used for the research were of the highest analytical grade. Basil seeds, produced by Burpee Garden Products Co (Warminster, PA), were purchased at a local Home Depot store. Garden soil, vegetables and herbs mix with fertilizers and pot plants were also purchased at a local Home Depot store in New Jersey.

2.2. Methods and Apparatus

To increase the germination rate of different plants, many studies have utilized cold plasma to treat their seeds [16, 19, 52, and 53]. This study went beyond the others by applying atmospheric cold plasma jet on the plant bodies such as stems (S), leaves (L), and flowers (F) with an increase in the time treatment frequency. The study was further focused on different parameters which were involved during the growth process (water uptake, humidity, temperature and pH of the fertilizers). This study can help to evaluate and classify a series of reactive species that can be produced due to a plasma effect. This practice would also help to understand the mechanism of their development as explained by plasma physics, chemistry, and biology [42].

To understand the above aspects of the application of cold plasma on sweet basil, our experiment was divided into different treatment groups: control group (no plasma treatment), seed treated plant

(only seeds were treated), body treatment once a week and plant body treatment twice a week (their seeds were treated with plasma), as described below.

2.2.1. Atmospheric Cold Plasma Jet (ACPJ) Treatment of Basil Plants

The experimental setup of this study uses a high voltage power supply to energize a hollow steel cylinder that serves as an electrode. The electrode is fitted into a Teflon® tube which serves as both the gas propagation vessel as well as the dielectric material required for proper discharge energetics—which is to say it transmits the high voltage electric charge. High-purity helium gas serves as the medium for the plasma generated in Figures 2a & 2b. The plasma effluent can then be easily manipulated to serve as the treatment source for the plants in the study. Basil seeds were treated with ACPJ following the protocol from the previous study [16].

Approximately 500 seeds were placed in a glass petri dish and hand rotated for five minutes as the plasma effluent from the ACPJ interacted with the seed layers from a distance of approximately two centimeters. The jet that used during the seed treatment is showed in figure 3. The plasma was formed with an applied electrical potential of 10 kV and at about 22 kHz frequency. The gas medium for the plasma was ultra-high purity helium and was fed via a Cole Parmer® mass flow controller at a flow rate of 5.00 standard liters per minute (SLM). After all seeds were treated with the cold plasma, the planting process was divided into four groups. All plant groups were grown in vegetable herb soil, and irrigated with tap water every other day from our lab.

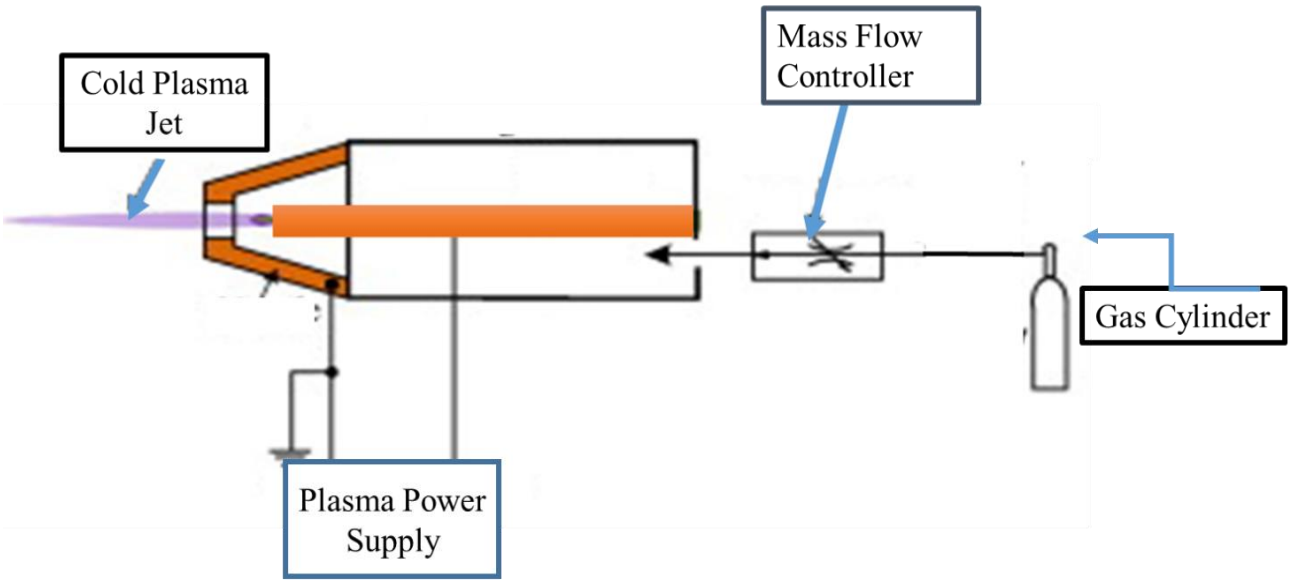


Figure 2a. - Apparatus of Atmospheric Cold Plasma Jet System

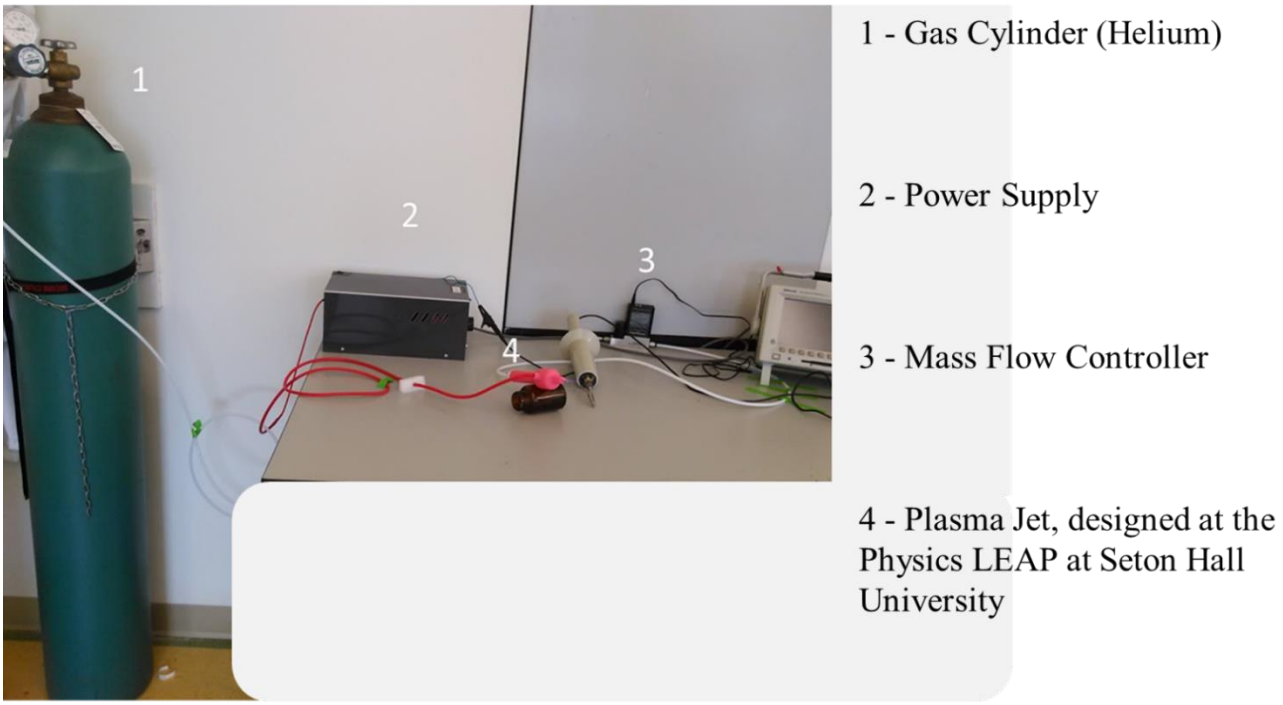


Figure 2b. - Atmospheric Cold Plasma Jet System used During the Experiment, Picture taken by the author during the experiment

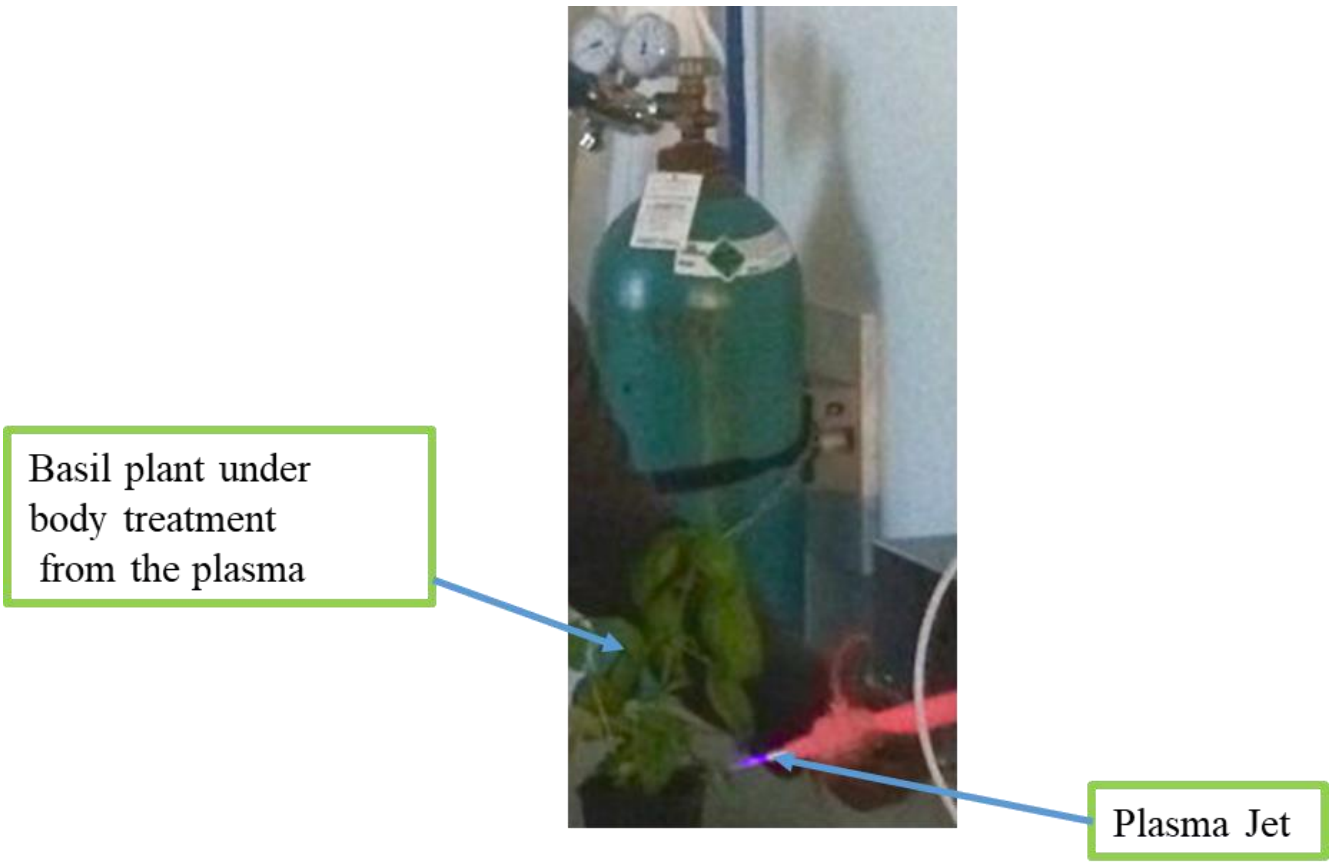


Figure 3. Image of the Body Treatment from Plasma Jet, Picture taken by the author during the experiment

Group A. Control group, seeds received no plasma treatment before planting.

Group B. Seeds treated with plasma before planting, no further plasma treatment.

Group C. Plasma seed-treatment as Group B. This group additionally receives plasma treatment immediately after germination. Germination is a process that leads to the sprouting of a seedling (young sporophyte develops from the seed embryo) from the seeds of the spermatophyte. Once germination occurred, a one-week rest period was given before the plant-body plasma treatment was initiated. The plant body (stems, leaves, and flowers) received cold plasma treatment once a week (1X/W) for thirty seconds on average**.**

Group D. Plasma seed treatment like Group C, however, plasma plant-body treatment was applied twice a week (2X/W).

As explained above, the plants were divided into four groups from control, seed treated, to body treatment once a week and body treatment twice a week (GA, GB, GC, and GD where "G" stands for group). Thirty seconds of the treatment were applied to the plants scheduled to receive body treatment either once a week (group C) or twice a week (group D).

2.2.2. Extracting the Essential Oil from the Plants

2.2.2.1. a. Soxhlet Extraction

After fourteen weeks of the growth period, the plants were harvested and subjected to a soxhlet extraction which, along with the steam distillation, is one of the techniques typically used to extract essential oil.

Soxhlet extraction is a solid/liquid extraction. It involves the transfer of partially soluble components of a solid to the liquid phase. The experimental set up for the soxhlet extraction includes a soxhlet extractor, electric mantle heater, water condenser, and flash evaporator.

The soxhlet system requires elevated temperature (^{o}C) which is determined by the boiling point of the solvent used.

Soxhlet extraction is a continuous solid/liquid extraction where the solid contains the material to be extracted. The solid is placed in a cellulose thimble, which is placed into the main chamber of the soxhlet extractor and the solvent is allowed to pass the siphon arm with continuous refluxing. The length of the soxhlet varies from 18 to 24 hours.

2.2.2.1. b. Extracting the essential oil from the plants, using soxhlet technique

After harvesting the plants from each group, the essential oil was extracted from different parts of the plants (leaves, stems, flowers and whole plants). The experimental matrix was divided into four groups, “GA” for control, “GB” for seed treated, “GC” for body treatment 1x/w, “GD” for body treatment twice a week with the code names for each extraction given as follows:

GAL, GBL, GCL, and GDL - extracts in this group derived from leaves (L),

GAS, GBS, GCS and GDS - extracts in this group derived from stems (S),

GAF, GBF, GCF and GDF - extracts from flowers (F), and

GAM, GBM, GCM and GDM – extracts from the entire plant mixture (M)

Hexane was used as the extraction solvent.

2.2.2.2. High Pressure Reactor Extraction

Experiments were carried out in a specially designed reactor system, which enables one to continuously monitor the progress of reactions, associated by gas evolution or gas uptake. The reactor system consisted of a standard 100 mL stainless steel PARR Autoclave and a volumetric module, shown in Figure 4. The reactor was of a design which allows the entire vessel to be removed from the stand as a complete assembly for either charging or product recovery. The reactor was equipped with a magnetically coupled drive with a permanent magnet for the inner rotor, to which the stirring shaft was attached. A detailed picture of the reactor head and available port attachments is shown in Figure 5. The first port, 1, accommodated a safety rapture disc intended to release pressure when critical level was exceeded. Port 2 was a combination port which held a liquid sampling and gas inlet valves. In our specific arrangement, the sampling valve was used for siphoning out the liquid product without the need of opening the reactor. A deep tube fitted with stainless steel frit at the tip of the tube allowed extraction of the liquid phase while keeping the samples in the reactor for use in subsequent reactions. Port 3 was a second combination port accommodating a pressure gage and a 1/8” stainless steel needle valve. The latter was used as a primary gas inlet port for initially purging and pressurizing the reactor and the port itself was connected to the volumetric section of the reactor system through a 1/8 “stainless steel tubing.

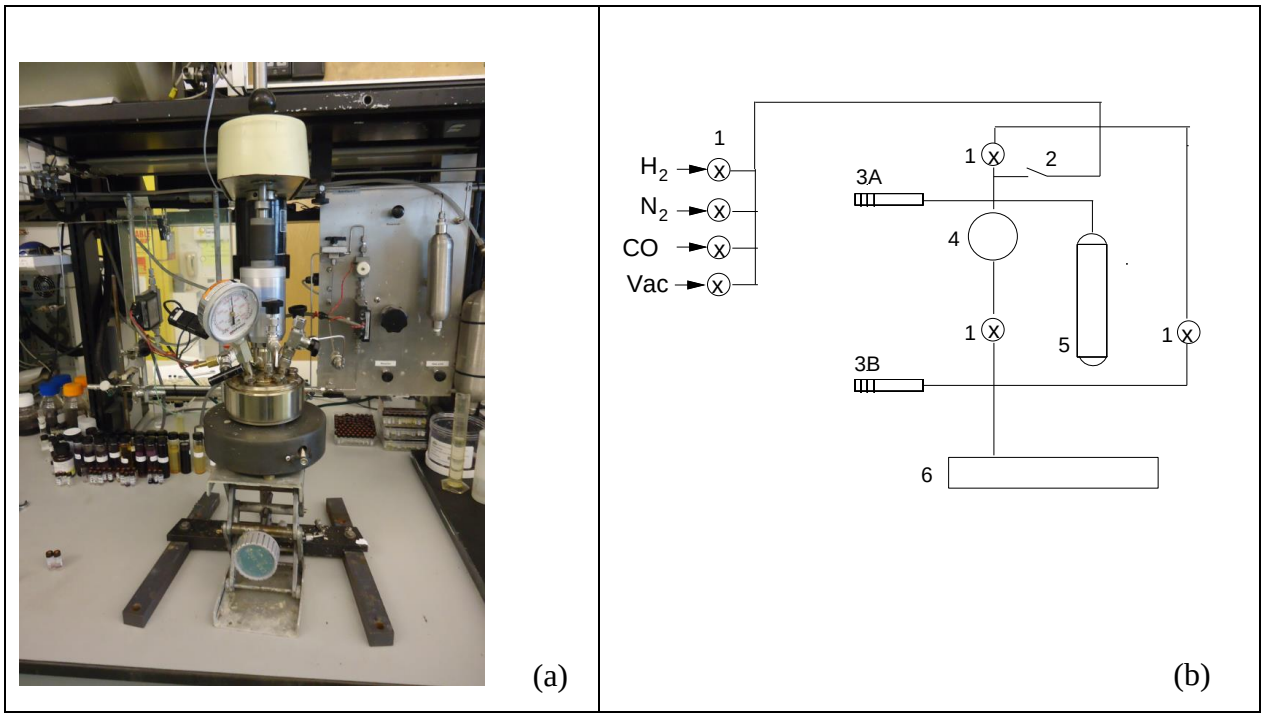


Figure 4. - Automated Parr Reactor (a) and Associated volumetric system (b) Picture taken by the author during the experiment

Port 4 was holding a J-type thermocouple while the remaining two ports can be fitted with a cooling coil for accurate control of the reactor temperature in cases when exothermic reaction was expected. In the current configuration, the cooling coil was removed to produce two additional ports. The first additional port is left blank while the second additional port, 6 was fitted with ¼" on/off valve used for loading the reaction solutions using gas tight syringes. A picture of the reactor is shown in the inset of Figure 4a. (Source: Parr Operating Instruction Manual, Parr Instrument Company, Moline, Illinois, USA)

The system was pressurized to appropriate pressure (300, 750 Psi), the reactor heated to the desired temperature (40, 70, and 100 ^{o}C) and each extraction step was routinely carried for 20 hours similar to soxhlet extraction at a stirring rate of 160 - 200 RPM. After this step, the reactor was cooled to ambient temperature and the content of the reactor was collected without filtering the extracted solution.

After each set of extraction, a rotary evaporator (model Büchi RE: 111) was used to remove the solvent and collect the essential oil for analysis.

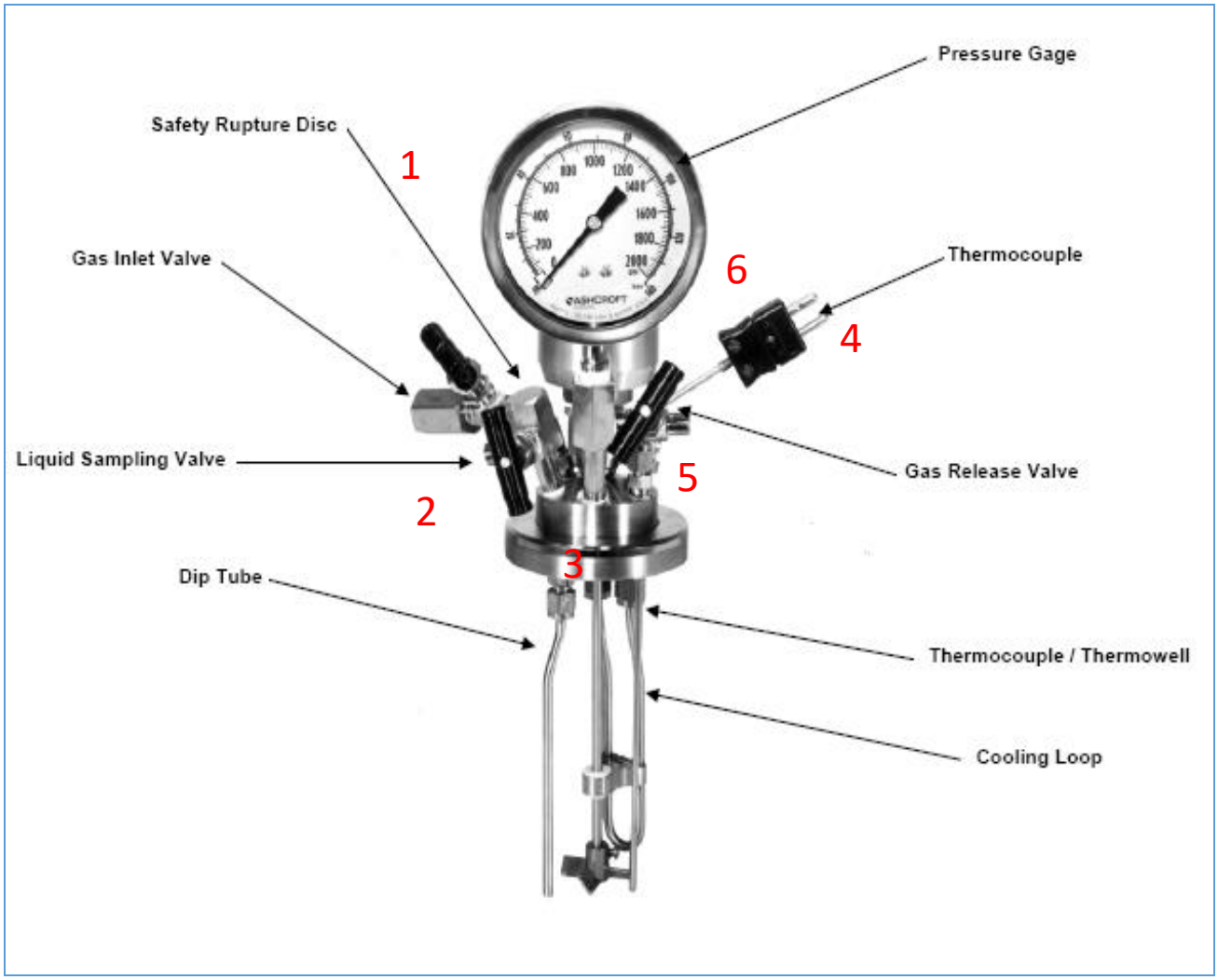


Figure 5. - Schematic of the Parr Reactor head
(Parr Operating Instruction Manual, Parr Instrument Company, 396M Series 4560 Operating Instructions, Moline, Illinois, USA, Picture taken by the author during the experiment)

2.2.3. Method Development for Quantitative Analysis of Basil Oil by Gas Chromatography (GC). Building Mass Spectral Library for Compounds Expected to be found in Basil Plant Extracts

In the current study, both GC/FID and GC/MS were used for qualitative and quantitative analysis of the essential oil extracts derived from the non-treated and plasma treated basil plants. The first system, GC/FID, was used for routine quantitative analysis. The system was fitted with an automatic sample injector and a data aquisition system allowing an uniterrupted analysis of a large number of samples. The system generated an area report file which was automatically exported to a compiled Excel spreadsheet via Dynamic Data Exhange Interface (DDEI) in which a spreadsheet was used to calculate the concentration profile for each component of interest. The second system, GC/MS, utilized in tandem with the GC/FID, was used mainly for identification of the compounds found in the plant extracts. Both systems employed the HP5890 apparatus and were operated at the same temperature program and used the same columns so that both systems produced the same retention times for each component of the complex mix. In cases when the routine GC/FID analysis revealed the presence of unexpected components, the parallel injection of the same sample in the GC/MS system allowed for an accurate recognition of the peak of interest by fitting the retention time and the mass spectra of the component of interest with those already available in the database.

2.2.3.1. Instrument Conditions

The GC/FID separation was carried out on a HP5890 Instrument fitted with HP7673 autoinjector and the system itself was interfaced to PeakSimple Chromatography Data system Model 333, manufactored by SRI Instruments. The GC/MS analysis was carried out on a HP5890 series II equipped with 5971A mass selective detector and MS ChemStation HP G103AMS controlling the both the GC and the MSD modules.

Both instruments were equipped with DB-624 fused silica capillary column (30 m x 0.25 mm ID, 1.40 μm film thickness) using a standard temperature program with initial oven tempertaure of 70^{o}C and ramp of 10^{o}C/min. Unfortunately, using this set of parameters led to a poor separation of limonene from the two isomers of ocimene. Increasing the initial temperaure to 90^{o}C improved significanty the separation and led to base line separation of these three components of interest. The settings for the two temperature ramp stages are as follows:

Ramp 1: Initial temperature 90^{o}C held 2 min, ramp 10^{o}C /min to 200^{o}C, hold 4 min
Ramp 2: 10^{o}C/min to 240^{o}C, hold 6 min. The injector and the detector temperature were held at 230 ^{o}C and 240 ^{o}C, respectively, with helium carrier gas flow rate of 40 cc/min at split ratio 39:1 and head pressure kept at 9 psi.

2.2.3.2. Reference Sample Preparation.

The model solution containing all potential reference compounds typically found in basil plant extracts was made by blending 100 µl of each of the 15 reference components from Table 1 into 10 ml of hexane. The concentration of each compound in this standard mix was calculated and listed in Table 2. Pentadecane was used as an internal standard in hexane with a concentration of 10 mg/ml. Prior to GC/MS analysis, the injection sample was prepared by combining 600 µl of the model solution with 600 µl of the internal standard solution. The retention time of pentadecane was not found to overlap with the retention times of the other basil components. A typical chromatogram of standard components is shown in Figure 6.

Table 2. Calculations of the Concentration of the Standard Blend Solution

Components	Volume initial	Density	Mass Calculate	Volume Solution*	Concentration
	µl		mg	ml	mg/ml
α-Pinene	100	0.858	85.8	11.5	7.5
Myrcene	100	0.791	79.1	11.5	6.9
β-Pinene	100	0.859	85.9	11.5	7.5
Limonene	100	0.841	84.1	11.5	7.3
Ocimene	100	0.818	81.8	11.5	7.1
Eucalyptol	100	0.922	92.2	11.5	8.0
Linalool	100	0.870	87.0	11.5	7.6
Camphor	100	0.990	99.0	11.5	8.6
Estragole	100	0.965	96.5	11.5	8.4
Terpineol	100	0.934	93.4	11.5	8.1
Citronellol	100	0.855	85.5	11.5	7.4
Geraniol	100	0.879	87.9	11.5	7.6
Eugenol	100	1.067	106.7	11.5	9.3
CAME	100	1.090	109.0	11.5	9.5
Pentadecane CAEE	100	1.050	105.0	11.5	9.1

*** The standard samples were prepared by mixing 1.5 mL of the oil blend with 10 mL of hexane into 10 mL stoppered graduated volumetric flask. The sample volume was maintained with refrigerated storage conditions.**

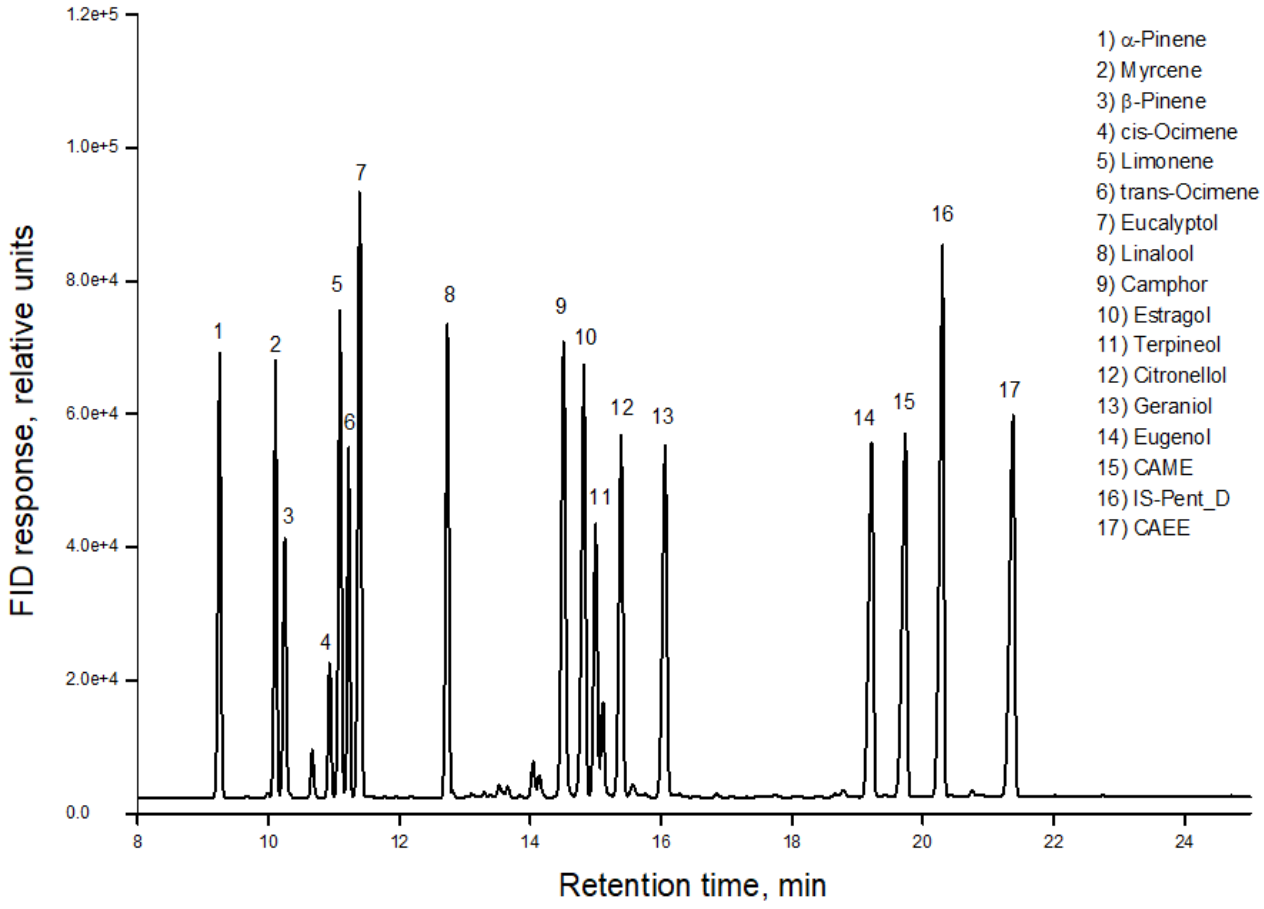


Figure 6. – Chromatogram of Standard Mixture of the Individual Component from FID System; Column used, DB-624; injection: 5µl; split ratio: 39:1; initial temperature: 90 ºC; holds 2 min; Ramp 10 ºC/min to 200 ºC holds 4 min, Program A: Ramps 10 ºC/min to 240 ºC hold 6 min; Injection and detector Temp: 230°C and 250°C

2.2.3.3. Extracted Oil Sample Preparation

Hexane was removed from the soxhlet extracts by rotoevaporation. The collected essential oil was re-dissolved in hexane, the solution was quantitatively transferred into a 5 mL volumetric flask and the final volume of the resulting solution was adjusted to the 5 mL mark with hexane. For the preparation of the GC sample prior to injection, again 600 µl of the sample solution was combined with 600 µl internal standard solution of pentadecane in hexane as described already in 2.2.3.2.

2.2.3.4. Determining the Response Factors (RF) for Each Component of the Oil Composition and Calculations for the Concentration Profile in Actual Oil Extracts

As already mentioned, both GC/FID and GC/MS were used in parallel for identification and quantitative analysis of the extracted samples. To achieve this, one needs to calculate the response factor for each of the identified components, including the internal standard [43,44,45]. The parameter RF as described by equation (1) below is usually calculated from a five level calibration for each component of interest by the slope and intercept of the resulting regression between concentrations and the resulting peak area derived from GC. In this particular study, given the time constraints to complete the work, the response factors were calculated in Table 3 by a single point calibration using a reference blend described in section 2.2.3.2.

$$\mathbf{Rf} = \frac{\frac{\mathbf{C_X}}{\mathbf{C_{IS}}}}{\frac{\mathbf{S_X}}{\mathbf{S_{IS}}}} \qquad (1)$$

Where:

Rf is the response factor, C_X and C_{IS} are respective concentrations of the unknown component and the internal standard, S_X and S_{IS} are the peak area of the same component and the internal standard. Re-arranging equation (1) into equation (2) allows calculation of the unknown concentration of the compound of interest based on the pre-determined Rf, the peak area ratio from the GC analysis and the concentration of the added internal standard.

$$\boldsymbol{C_X = C_{IS} * Rf * \frac{S_X}{S_{IS}}} \qquad (2)$$

This approach was used to program an Excel spreadsheet to calculate both the response factors for the 16 compounds of the reference blend and the concentration of the compounds found in actual

plant extracts before and after particular plasma treatment (for calculations, see Table B1 and B2 given in Appendix B).

Table 3. Response Factor Calculated from Area Report of the Standard Mix

Components	Sx (components)	S (IS)	Sx/S_{IS}	*RF = (Cx/Cis)/(Sx/Sis)
a-Pinene	51.627	122.260	0.422	1.77
Myrcene	47.370	122.260	0.387	1.78
b-Pinene	33.347	122.260	0.273	2.74
Limonene	60.175	122.260	0.492	1.49
Ocimene	53.670	122.260	0.439	1.62
Eucalyptol	87.263	122.260	0.714	2.05
Linalool	61.867	122.260	0.506	1.50
Camphor	78.200	122.260	0.640	1.35
Estragole	75.900	122.260	0.621	1.35
Terpineol	37.740	122.260	0.309	2.63
Citronellol	62.838	122.260	0.514	1.45
Geraniol	69.013	122.260	0.564	1.35
Eugenol	75.042	122.260	0.614	1.51
CAME	76.084	122.260	0.622	1.52
Pentadecane	122.265	122.260	1.000	1.00
CAEE	84.891	122.260	0.694	1.32

* These numbers are calculated from the area report from the second OB14 (abbreviation of the mixture) test mixture. For method verification purposes, the OB14 test mix was kept in a refrigerator and re-analyzed frequently to verify the consistency of the response factors for each components.

Chapter 3

Results and Discussion

According to the previous pilot study, cold plasma treatment has been found to increase the growth of sweet basil plant [16]. To investigate this effect, we planted and harvested sweet basil in our lab where the main focus was to investigate the effects of cold plasma treatment on sweet basil using different types of treatment: control [not treated (GA)], seed treated (GB), body treatment once a week (GC), and body treatment twice a week (GD). After the treatment of the seeds, all seeds changed color from black to purple. Right after the ACPJ treatment, the seeds were directly planted in the area designed for the experiment. The focal point of the study was on the growth of the basil plants and the physical effects of the ACPJ on the plants before and after the treatment. Chemical analysis was conducted on the extracted essential oil components.

3.1 – Growth of the Basil Plants under ACPJ Treatment from Germination, Vegetation, and Flowering

The results show effects of the plasma treatment on the plant growth in Figure 7. A week after the germination, plasma treatment was applied to the group set to receive treatment once a week (1X/week) and twice a week (2 X /week). Later, some plants in both groups died due (what we speculate to be) too early of an application of the plasma. This statement was supported by the similar deaths in the group set to receive plasma treatment once a week (1X/week), but at a lower rate. However, more death was observed in the group that received treatment twice a week and reduced the plant growth during the first five weeks of the treatment as compare to the control and seed treated plants. The issue of plant death led us to investigate other biologic issues such as lack of hormone (auxin) or damage of salt bridge that facilitates water and nutrient uptake from the roots (aquaporin) [46]. Aquaporin is describe as the water channel of the cell plant, which is to facilitate water in and out of the cell and preventing the passage of ions and other solutes to the cell plants. Given a rest period from plasma treatment, the plants recovered quickly and grew faster in the last two weeks than the controls. Since then, they became more robust, stronger in their stems and larger in their leaves compared to the control group.

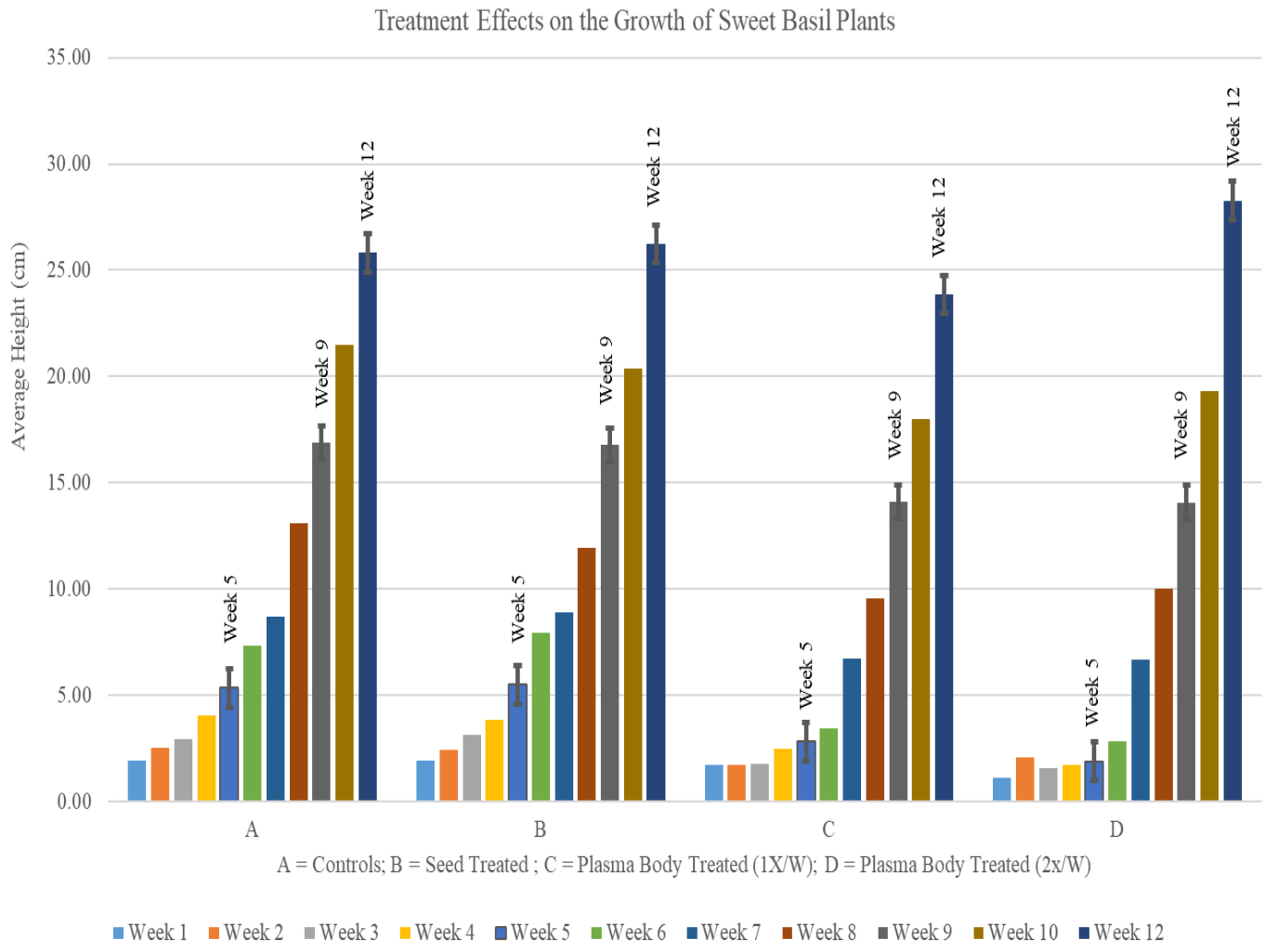


Figure 7. - Treatment Effects on the Growth of Sweet Basil Plants

Figure 8 showed the approximately average surface area of the leaves measured at the end of the growth. The plants treated with ACPJ showed growth increase in their leaves and stems throughout the study especially in the last week of the measurement. While plants grew, physical observations showed the leaves and the stems became stronger, larger, and more robust in the plant that received double treatment with the plasma than the control. The length and width of the leaves were measured in randomly selected groups of ten plants from each group where the lengths were measured from the stem to the leaf tip and the width across the middle of the leaf and the approximate average surface area (**S =** length * Width) was calculated.

The determination of the surface area shows a clear effect in the plant groups that received plasma body treatment twice a week compared to the control group. The group that received double treatment grew faster in height in the last week as shown in Figure 8 and Figure 9 as well as in their leaves. Also, the plants required more water, their leaves became larger and greener as they grew up.

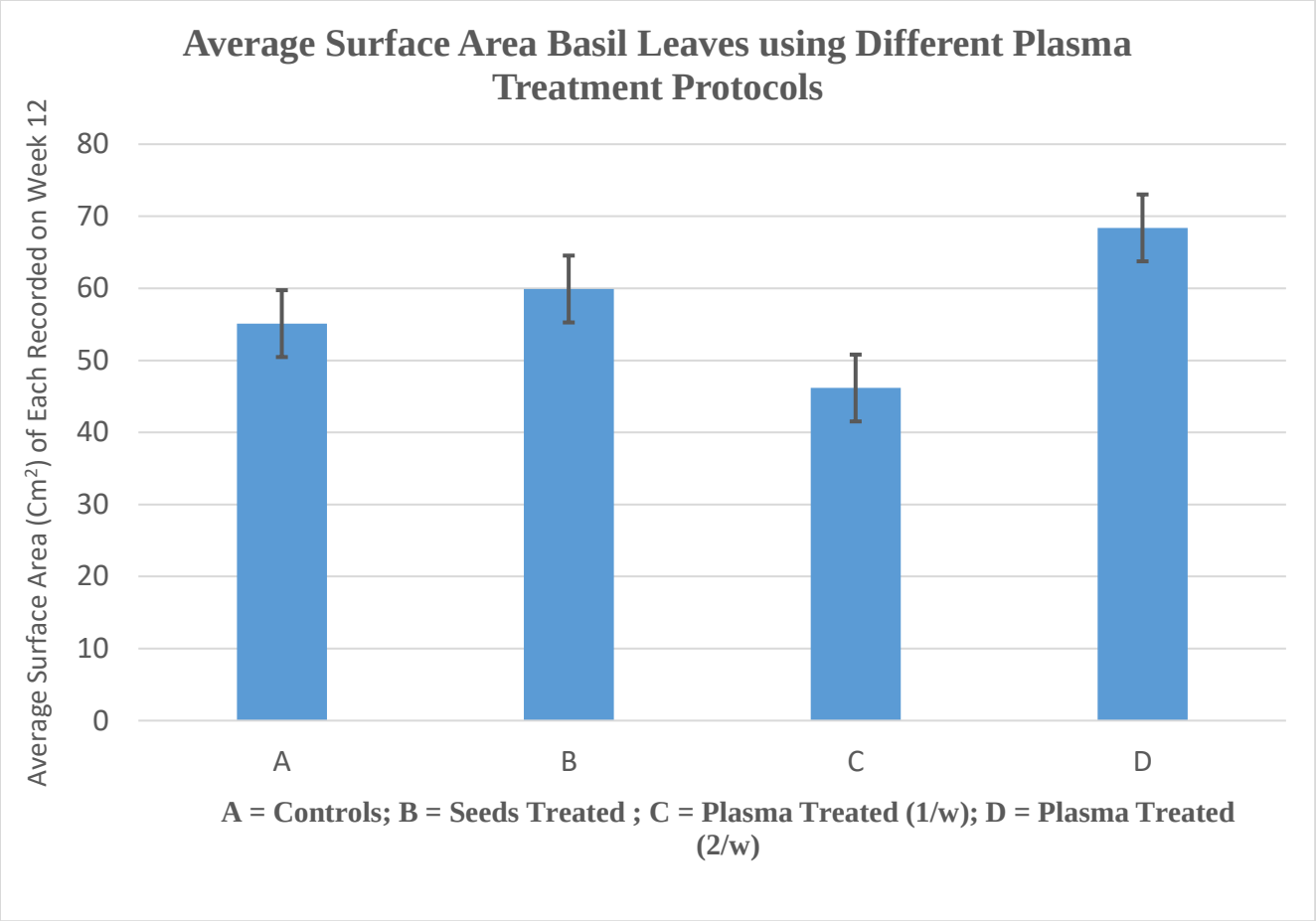


Figure 8. – Measuring the Average Surface Area of the Leaves from Control to Plasma Treated Plants on Week 12

Figure 9. – Photos of basil plants and the physical growth. (A): Control; (B): Seeds treated with Atmospheric Cold Plasma Jet (ACPJ); (C): Seeds Treated and body treatment once a week (1X/W), and (D): Seeds Treated and body treatment twice a week (2X/W)

3.1.1. - Biological Aspect of the Growth Using ACPJ Treatment

Throughout the plant growth, different biological aspects were detected during the plant cycle. One of the most important aspects was nutrient uptake via water coming from the plant roots. As the plant received plasma treatment, the moisture of the soil was measured with a soil moisture meter placed on each pot of the plant where the moisture soil was recorded. More water was needed by the plants that received plasma treatment as compared to the control because the soil became less moist than the control.

As the plants received plasma treatment at the early stage, they suffered stress and displayed yellow to brown discoloration from leaves to stems, and later the plants died.

Applications of the cold plasma treatment at the early stages of vegetation might be detrimental to the plant's cell walls [47]. Since the cell wall is affected by the different stage of the plant, the early application of the plasma treatment might cause a negative impact on different parts of plants cell walls. Consequently, after the plants started their recovery from plasma treatment, certain physical observations potentially occurred in terms of biological, physical, and chemical effects. These effects occurred in the plants which facilitated better transport of water and nutrients from the roots to other parts of the plants. Similar studies have shown that plasma treatment produces positive results in certain foods, vegetables, fruit, and meat products [48].

3.2. – Qualitative Analysis of the Extracted Essential Oil Components

The concentrations of the extracted components varied between the control and the treated samples. Figure 10 consists of four vertical panels labeled A1-A3 (control), B1-B3 (seed treated), C1– C3 (once a week treatment), and D1 – D3 (twice a week treatment). The panels show the sample extracts from each part of the plant, i.e., leaves, stems, and flowers.

Different components, extracted from leaves and flowers show some similarity in composition, however they vary in relative amount in each group. In contrast, extracts from the stems contained insignificant amounts of components of essential oil.

Effect of Cold Plasma on Different Parts of the Plants

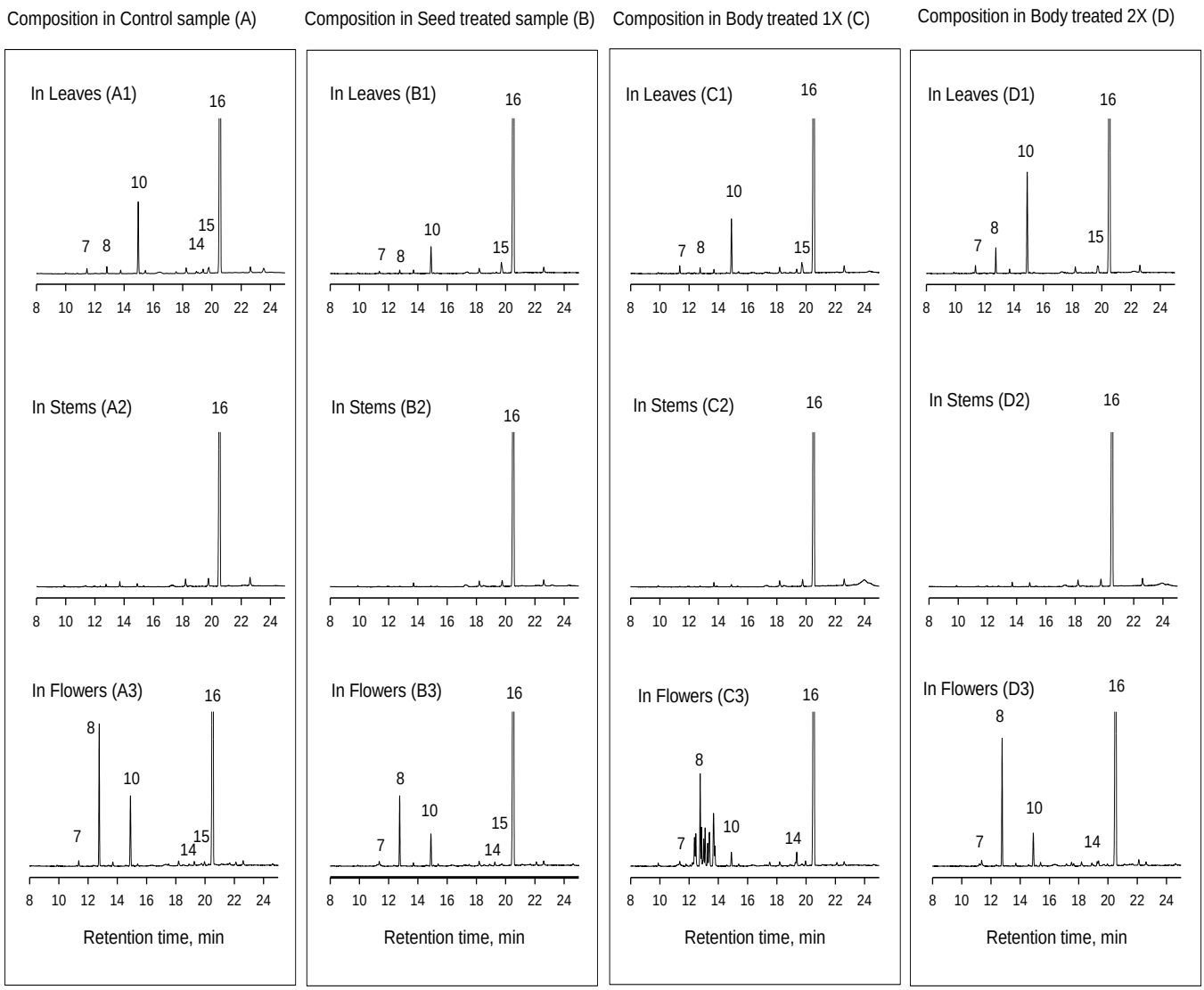


Figure 10. Composition of the Basil Oil from the control group (A) and Plasma treated groups (B - D) Recovered by Soxhlet extraction with hexane.
Components identified in order according to the standard mix, Eucalyptol (7), Linalool (8), Estragole (10), Eugenol (14), CAME (15). Internal standard pentadecane (16)

The GC profile of the oil extracts recovered from various parts of the plant clearly shows that (a) there are some significant differences in the product distribution in the leaves and in the flowers and (b) the ACPJ treatment had effect on the level of estragole and linalool formed in each group of the experimental matrix. The composite data from Figure 10, representing the product composition in extracts collected from various parts of the basil plants, can be summarized as follows.

Product Distribution in Leaves (**Figures 10 A1, B1, C1, & D1**)

In extracts from leaves, estragole, **10,** was found to be dominant in the control group, it decreased in seed treated, increased in the body treatment once a week, and continued to increase in the "body treatment twice a week" groups. In addition, linalool, **8,** in the leaves was found in relatively low amount in the control, seeds treated and "once a week" treatment, while it increased in the double treatment group. Surprisingly, eugenol, **14,** was not found in the leaves from seed to double treatment, but it was found in minor amount in the control. Eucalyptol, **7,** was detected in relative low concentration in both the control and in the seed treated group while it increased in the body treated plants. Overall extraction from the leaves showed five components of the essential oil in the control group: eucalyptol, linalool, estragole, eugenol, and cinnamic acid methyl ester (CAME). Similar composition (except eugenol) was routinely detected in plasma treated groups. In general, linalool and estragole are the two dominant components present in the leaf extracts in both the control and in the plasma treated plant groups.

Product Distribution in Stems (F**igures 10 A2, B2, C2, & D2**)

Extraction from the stems did not show any significant presence of components in control and treated plants. Stems are used to support the plants and transport nutrients and water from the roots throughout the different part of the plants. Since a major role of the plant stems is to facilitate the transportation of water and nutrients to other parts of the plants, the stems do not have a large amount of the components as other plant parts. In most plants the stems are exposed aboveground, however in some types of plants they are hidden below the ground. The stems of the sweet basil are exposed above ground and have been defined as a "central axis" to which all other parts of the

plants are attached [49]. In the growth of the plant, the stem accomplishes the role of storing and transporting nutrients for photosynthesis.

Product Distribution in the Flowers (F**igures 10 A3, B3, C3, & D3**)

Compared to the leaves and the stems, the essential oil components isolated from flowers are present in relative higher amounts than in the other parts of the plants. Linalool, **8,** is predominant in the flowers with a higher amount in the control, less in seed treated, with an increase in once and twice a week treatment protocols. Once a week treatment in the flowers (C3) showed some complexity in the analysis due to contamination during the extraction process. Similar to linalool, estragole, **10,** was found in a higher amount in the control, less in seeds and once a week treatment, with an increase in double body treatment. Eugenol, **14,** was found in the body treatment (1X/W) to be at the highest level among the other groups extracted from the flowers comparable to first preliminary study. Note that compared to the leaves, five components of essential oil were found in the flowers: eucalyptol, linalool, estragole, eugenol, and CAME. CAME, **15,** was not identified in the two body treatment groups (1X & 2X) while linalool and estragole were the two dominant components of the essential oil found in the flowers.

3.3 - Quantitative analysis of Essential Oils Recovered from Various parts of the Plants in the Control and in the Plasma Treated Groups.

The response factor was used to calculate the concentration of each component determined by the analysis. Equation 2 (section 2.2.3.4) was used to calculate the absolute concentration of each component based on its peak area. Table 4 shows the concentration of the compounds extracted from each part of the plant, including the total of the entire plant (“mixture”). For ten grams of fresh basil leaves, the highest concentration (1.579 mg/ml) was observed in the double body treatment, Figure 11a. For example, for ten grams of fresh leaves, estragole concentration was 1.071 mg/ml, 0.394 mg/ml, 0.826 mg/ml, and 1.579 mg/ml, respectively, for the control group, seed treated, body treatment once a week, and body treatment twice a week groups in Table 4A. Estragole was found with the highest concentration in the leaves, as shown in Table 4B and Table B2 in the Appendix B.

Table 4A. Concentrations (mg/ml) of the Extracted Basil Oil Components for Various Part of the Plants

Components	GAL	GBL	GCL	GDL	GAS	GBS	GCS	GDS	GAF	GBF	GCF	GDF
α-Pinene	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Myrcene												
β-Pinene												
Limonene												0.027
Ocimene												
Eucalyptol	0.104	0.046	0.158	0.159	0.000	0.000	0.000	0.000	0.115	0.117	0.108	0.127
Linalool	0.089	0.045	0.078	0.345	0.035	0.000	0.000	0.000	1.979	0.943	1.131	1.766
Camphor	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Estragole	1.071	0.394	0.826	1.579	0.053	0.000	0.040	0.065	1.113	0.486	0.219	0.522
Terpineol												
Citronellol												
Geraniol	0.110											
Eugenol	0.064	0.020	0.068	0.000	0.000	0.000	0.000	0.000	0.087	0.075	0.209	0.055
CAME	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.072	0.000	0.091	0.042
Pentadecane	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
CAEE									0.073			0.019

* The assignment of the above symbols are previously described and explained in section 2.2.2.1.b. of the method.

4B. Composition of the Basil Oil Extracts Derived from Various Sections of the Plant after Plasma Treatment by the Standard Protocol (mg

Components	GAL	GBL	GCL	GDL	GAS	GBS	GCS	GDS	GAF	GBF	GCF	GDF	GAM	GBM	GCM	GDM
a-Pinene																
Myrcene																
b-Pinene																
Limonene												0.135				
Ocimene																
Eucalyptol	0.522	0.232	0.791	0.797	0.000	0.000	0.000	0.000	0.575	0.586	0.542	0.633	0.940	0.313	0.390	**0.665**
Linalool	0.445	0.224	0.391	1.726	0.174	0.000	0.000	0.000	9.893	4.714	5.655	8.829	3.523	1.724	2.021	**2.402**
Camphor																
Estragole	5.354	1.971	4.130	7.897	0.265	0.000	0.200	0.323	5.566	2.430	1.096	2.609	3.047	4.091	4.443	**3.500**
Terpineol																
Citronellol																
Geraniol	0.552	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
Eugenol	0.322	0.099	0.342	0.000	0.000	0.000	0.000	0.000	0.434	0.375	1.043	0.274	0.545	0.000	0.299	**1.474**
CAME	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.361	0.000	0.457	0.209	0.128	0.000	0.111	**0.000**
Pentadecane																
CAEE	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.365	**0.000**	**0.000**	0.094	**0.000**	**0.000**	**0.000**	**0.000**

Note: the description of the above symbols are previously explained in section 2.2.2.1.b. of the method.

In the distribution of the stem extraction, no significant essential oil was identified, Figure 11b. In the flower extraction, linalool and estragole were predominant with the highest concentration from five grams of fresh flowers extracted. Linalool was found to be higher in concentration from control to the treatment, 1.978mg/g, 0.942mg/g, 1.13mg/g, and 1.766mg/g, respectively. Second to linalool in concentration was estragole. In the flower, the concentration decreased in the seed treated and increased in the body treatment groups, shown in Figure 11c and Table 2.

When it comes to total plant extraction (M), the concentration of the essential oil components calculated could not be compared to the leaf and flower extraction due to non-proportionality mass of each part on the plants, as shown in Figure 11d.

Over all, five components of the basil essential oil were identified in the study: eucalyptol, linalool, estragole, eugenol, and cinnamic acid methyl ester (CAME). Based on the previous study, estragole and eugenol are two main essential oil components that are effective food preservatives and can potentially replace synthetic antioxidants, butylated hydroxyl-anisole (BHA) and butylated hydroxyl-toluene (BHT) [16].

The pilot study found an increase of eugenol in the plasma-treated plant compared to the commercial basil oil that contains linalool and estragole as the main essential oil components [16]. The pilot study used steam distillation for the extraction technique. However, the present study found five main components from control to treatment with a potent difference in their concentration by using soxhlet extraction, as shown in Figure 11. Further research will be helpful to compare the two extraction methods.

Effect of Cold Plasma Treatment on Sweet Basil Plant under Soxhlet Extraction

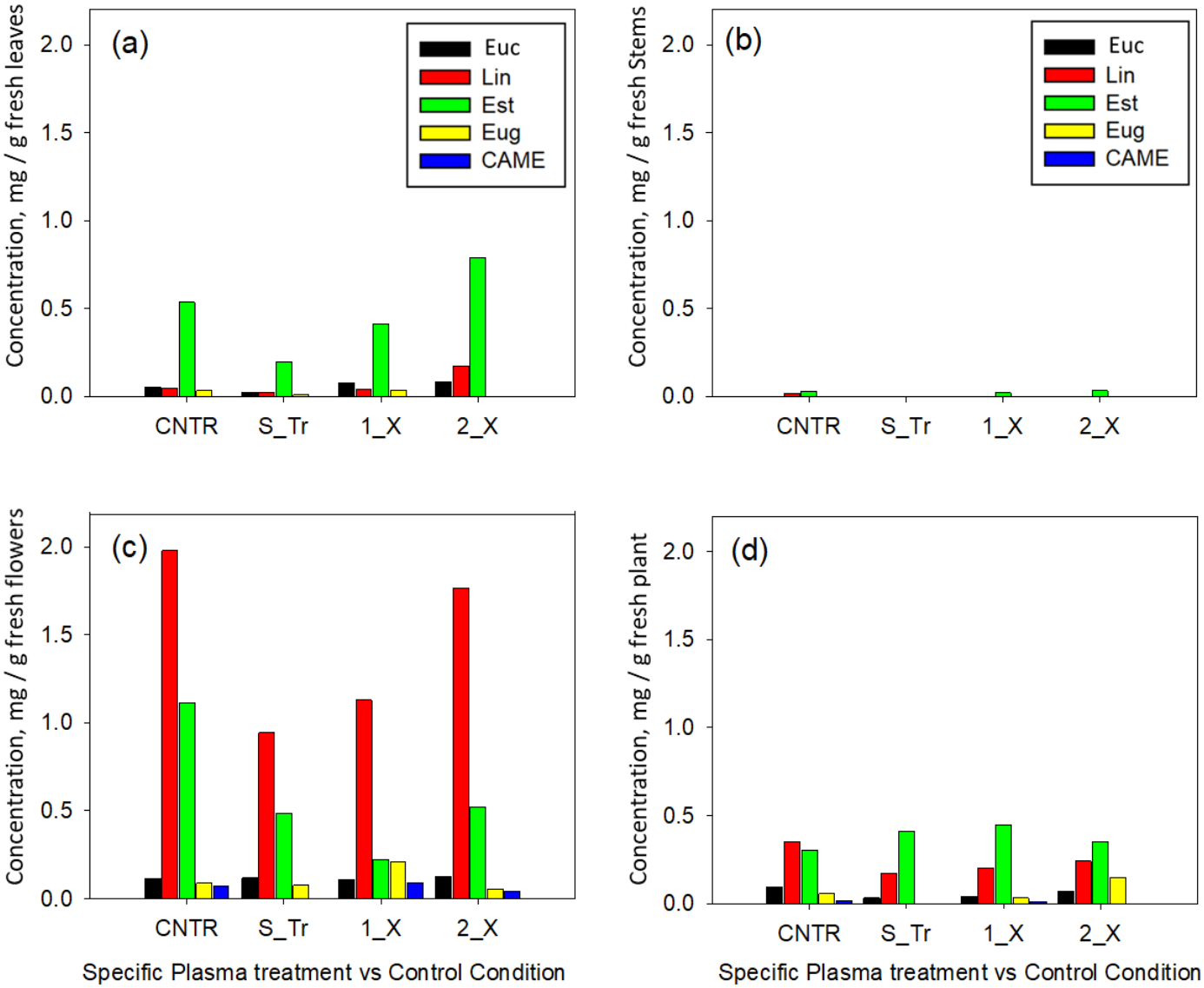


Figure 11. – Effects of cold plasma on each component found in the extracted plant: a) leaves, b) stems; c) flowers, and d) extraction of the whole plant with unequal amounts of each part of the plant.

3.4 - The Effect of Temperature and Pressure on the Extraction of Basil Plant Essential Oil using a High-Pressure Reactor

To look for a better recovery of the extraction of essential oil over soxhlet extraction, a high-pressure reactor was used with approximately three grams of dry basil. The extraction was set at three different temperatures and pressure conditions. The time of the extraction was similar to soxhlet extraction. Results are similar between the two techniques since five components were identified in both: eucalyptol, linalool, estragole, eugenol, and CAME shown in Figure 12a. However, the results showed the higher the temperature the higher the concentration of the components. In the high-pressure reactor extraction, estragole was found at the highest concentration, followed by linalool, eucalyptol, cinnamic acid ethyl ester, and eugenol, shown in Figure 12b.

Effect of the Temperature and Pressure of the Extraction System

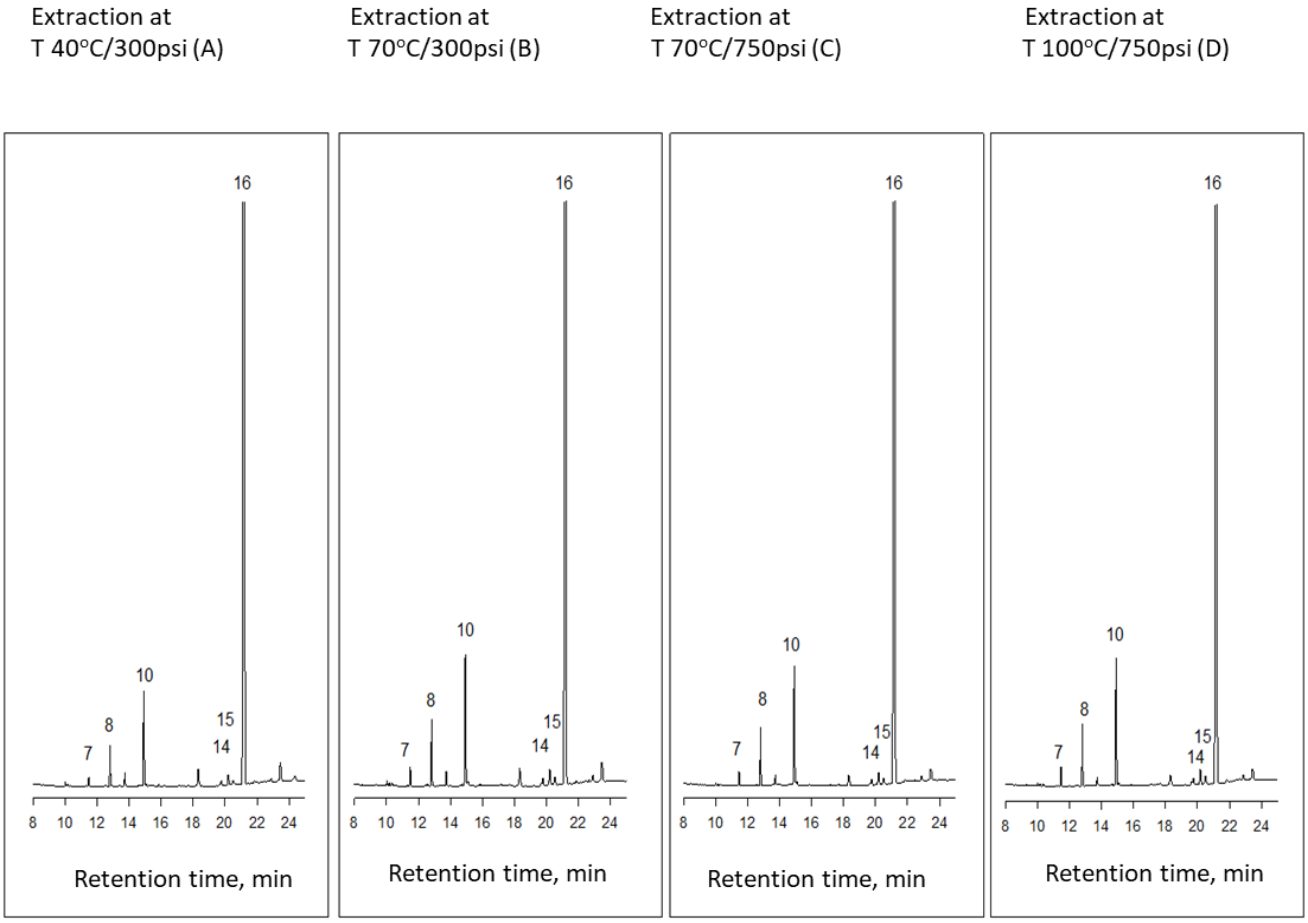


Figure 12A. Effect of the temperature and pressure on the extraction of sweet basil oil.
Eucalyptol (7), Linalool (8), Estragole (10), Eugenol (14), CAME (15), and internal standard of Pentadecane (16)

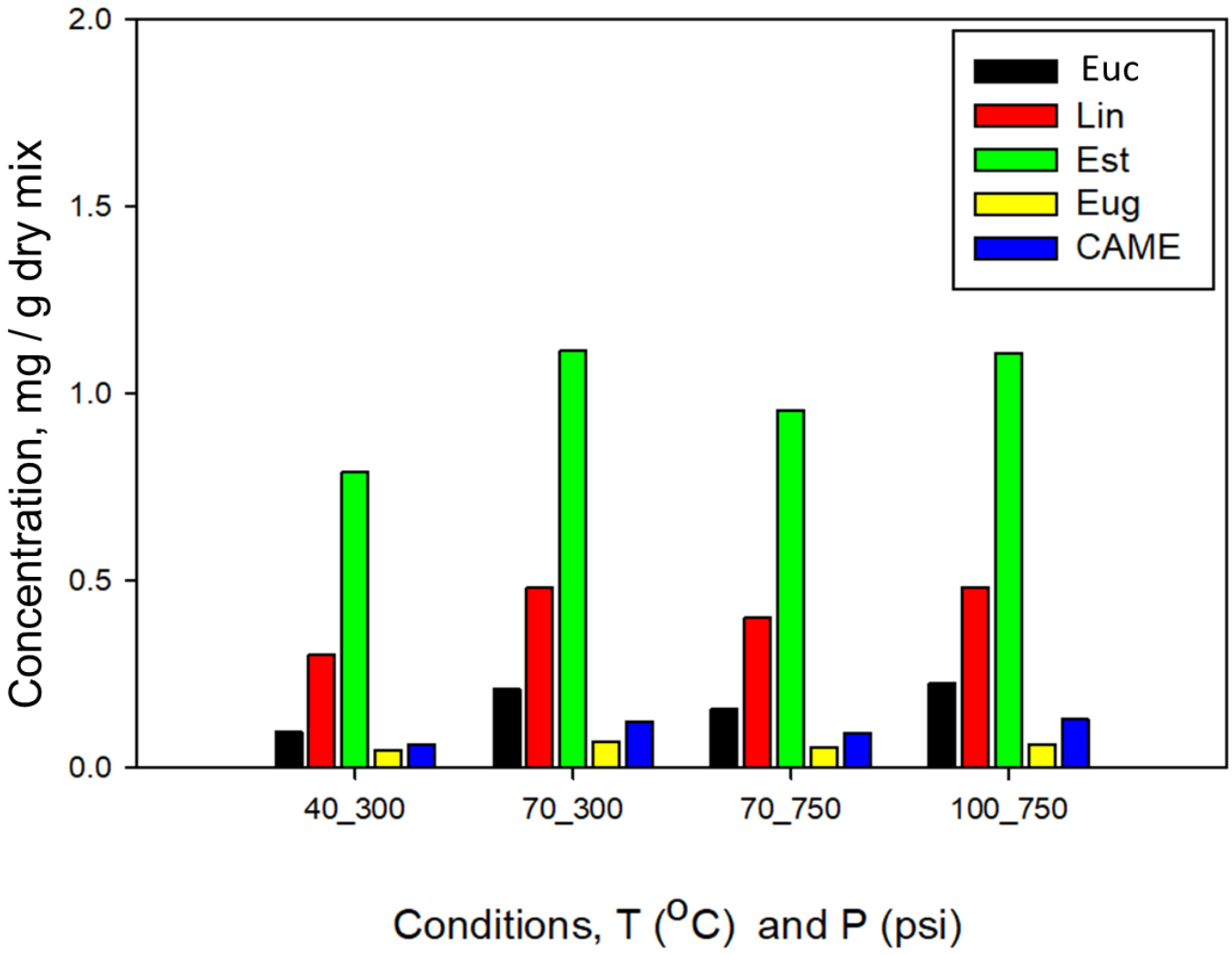


Figure 12B. – Concentration of the main components found as a function of the temperature and pressure of the extraction

The above graph in Figure 12b shows results of the effect of temperature/pressure under different conditions from 40 to 100 ^{o}C and 300 to 750 Psi. Under high pressure (300Psi) and low temperature (40 ^{o}C), low concentration of the components was found. As temperature increased, the concentration increased and dropped with an increase in the pressure. An increase of the essential oil components was identified by using high pressure reactor condition.

3.5 Analysis of the Effects of Atmospheric Cold Plasma Jet

The above results reveal certain questions on the application of the cold plasma. What caused the effects of atmospheric cold plasma in the plants? Since plasma is composed of equal numbers of positively charged ions and negatively charged electrons, which plasma components are responsible for the plant's growth?

The mass flow controller indicated that gas velocity of the helium did not exceed five liters standard per minute (LSM) from the plasma jet, Figure 2b. With 5 LSM the flow speed could not affect the plants as compared to the previous study, which used the same mass flow controller [16]. During the plasma treatment, we did not see immediate effects, the observed effects happened slowly throughout the growth of the plants.

In addition, to evaluate the effects of cold plasma radiation, randomly selected plants from each group were placed next to the discharge tube, where visible and UV radiations of the plasma jet both have maximal values. There was no effect at the surface of the plants, which was observed during the particular treatment. The contact of the jet to the plants might create some inner reaction, which is not visibly observed right away, Figure 13.

Figure 13. - Image of the body treatment at the surface of the plant leaves, Picture taken by the author during the experiment

Physically, to have a good understanding of the effects of electric fields and ions of the cold plasma in the plants, we have kept approximately the same distance between the plant surface and the jet exit. Past research with plasma jets using argon or helium gas have shown ionization streamers traveling with velocities in the range of 10^4–10^6 m/s that are orders of magnitude faster than the gas flow velocities [50]. The highest densities of electrons and ions inside an argon plasma measured under similar conditions with helium plasma have maximal values of 10^{18} m^{-3} and electron temperature does not exceed 4.5 eV (electron-volt) [51, 52]

Daily observations were made and weekly measurement were taken to improve the effects of the plasma. A drastic change was observed in the plants during the last stage of vegetation due to plasma treatment: growth increased in the stems and leaves where plants required more water daily, Figures 7-9. These changes would later clarify our understanding after the plants were extracted and analyzed by the GC. In this study, the components of essential oils were found at different concentrations from control to seed treated and body treatment, Figures 11, 12. Therefore, the applications of the atmospheric cold plasma jet treatment in the plant stimulate, and speed up the plant growth at a certain rate. In addition, plasma stimulates the production of the essential oil components.

Research conducted by others such as Thirumdas et al., demonstrated that: “Plasma treatment facilitates the release of bound phenolic compounds during extraction processes, which in turn increases antioxidant properties. Therefore, it can be concluded that the use of low temperature plasmas, a novel technology in grain processing, not only maintained the high nutritional values of basmati rice flour, but also provided better functional properties” [53]. This particular research on cold plasma treatment of flour and the work presented in this Thesis on sweet basil and its essential oils are both clear evidence that using cold plasma treatment on botanicals holds much promise in agriculture and food production and processing.

Chapter 4

Conclusions

The present investigation on sweet basil showed important effects of ACPJ treatment on sweet basil. Results show that the concentration of the essential oil components extracted from different treatments depends on treatment intensity. Estragole was found to be in the highest concentration in the leaves as compared to the flowers. Linalool had the highest concentration in the flowers followed by estragole, eugenol, eucalyptol, and methyl cinnamate. Results show that the highest concentration of the essential oil found in the plant, either the leaves or flowers, are based on the treatment time. The incorporation of the additional treatment groups in this study compared to the previous pilot study provides a clearer picture of the chemical and physical effects of plasma processing on basil and potentially in other botanicals.

Sweet basil cultivated in the lab was found to have a high content of essential oil, whose dominant component in the leaves was estragole. In addition this work found that geraniol, eucalyptol, linalool, and eugenol were found in larger amounts in the leaves. The highest amount of essential oil found in the flowers was linalool, followed by estragole, eucalyptol, eugenol, cinnamic acid ethyl ether, and CAME.

The identification of the essential oils was marked by a low proportion of eugenol, which varied from 0.099mg to 1.043mg as compared that reported in the pilot study [16], which showed a higher eugenol concentration. The natural essential oils are valued because they can be used as food preservatives and have medicinal applications. The present study on sweet basil has shown that the cold plasma treatment significantly affects the plant growth and the products found in the essential oil extracts.

The main components in the leaves were found to be the estragole and linalool. In the flowers, linalool was the predominant compound found to be nearly 20 fold higher in concentration followed by estragole and in significantly lower level, eugenol, eucalyptol, and methyl cinnamate.

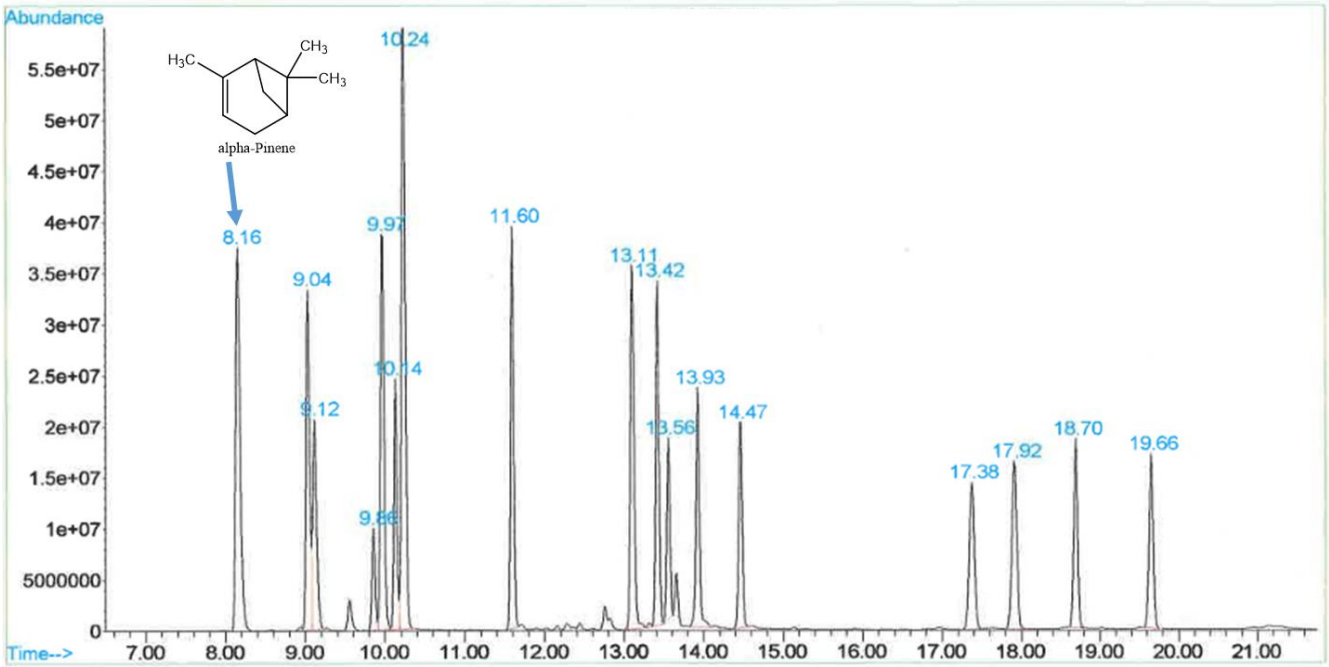
The most surprising results from the double-frequency plasma treatment was the significantly higher level of estragole and linalool isolated from the leaves. The same double-frequency treatment had little effect on the product distribution in the basil flowers.

In addition this study demonstrated that soxhlet extraction, a high pressure reactor approach improved extraction efficiency. With high pressure, the product profiles remained the same as soxhlet, however high pressure resulted in higher yield. However, future investigations might further focus on the different extraction techniques of essential oils, such as accelerated solvent extraction (ASE) and high temperature/pressure condition as these have not been investigated and might demonstrate improved extracted of essential oils from plants treated with cold plasmas.

Appendixes

Appendix A. –Mass Spectra of Individual Compounds Potentially Present in Basil Plant Extracts

Figure A1. Chromatogram of alpha-Pinene Standard



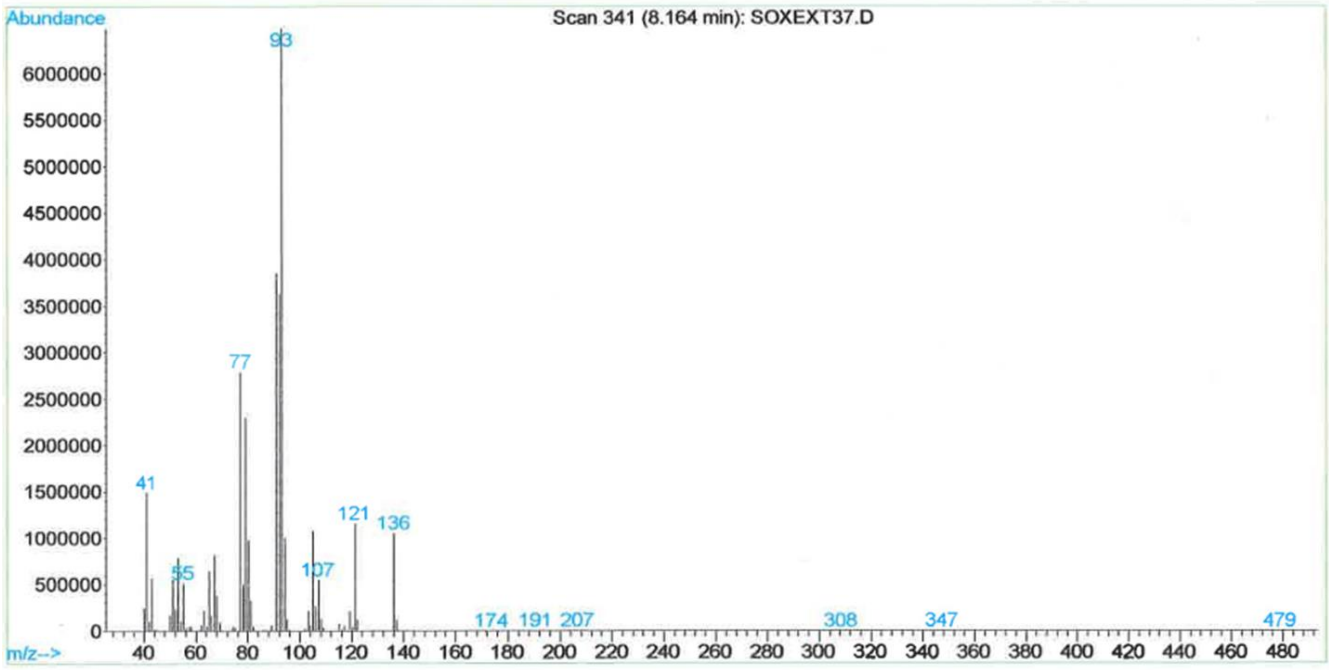


Figure A2. Chromatogram of Myrcene Standard

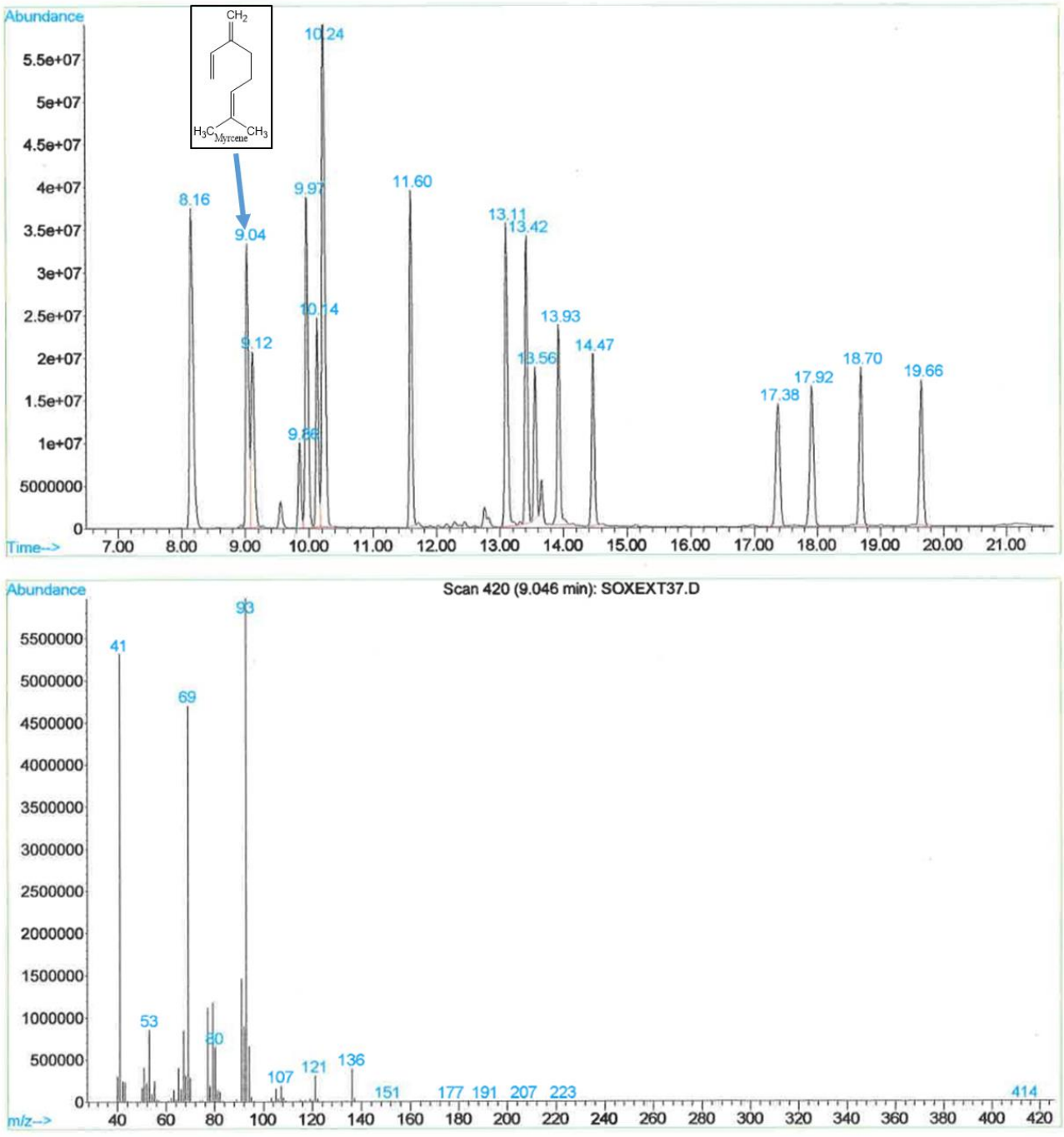
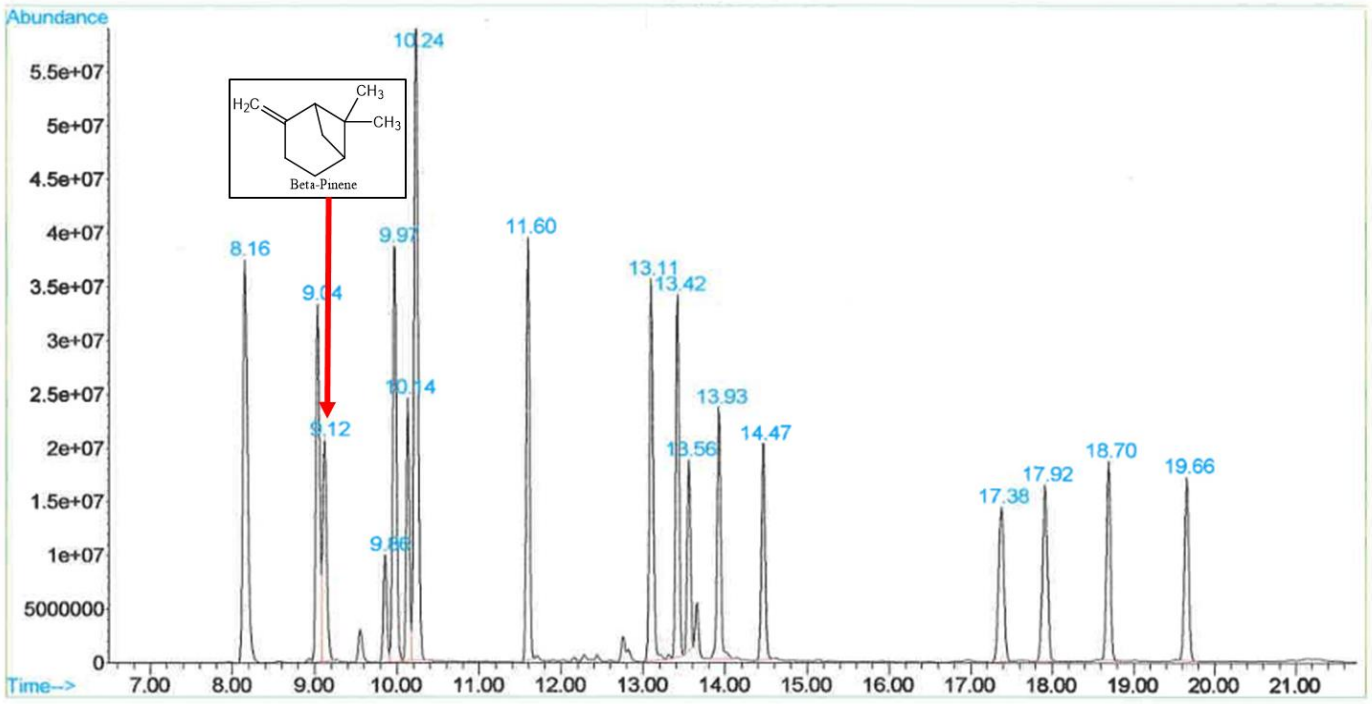


Figure A3. Chromatogram of beta-Pinene Standard



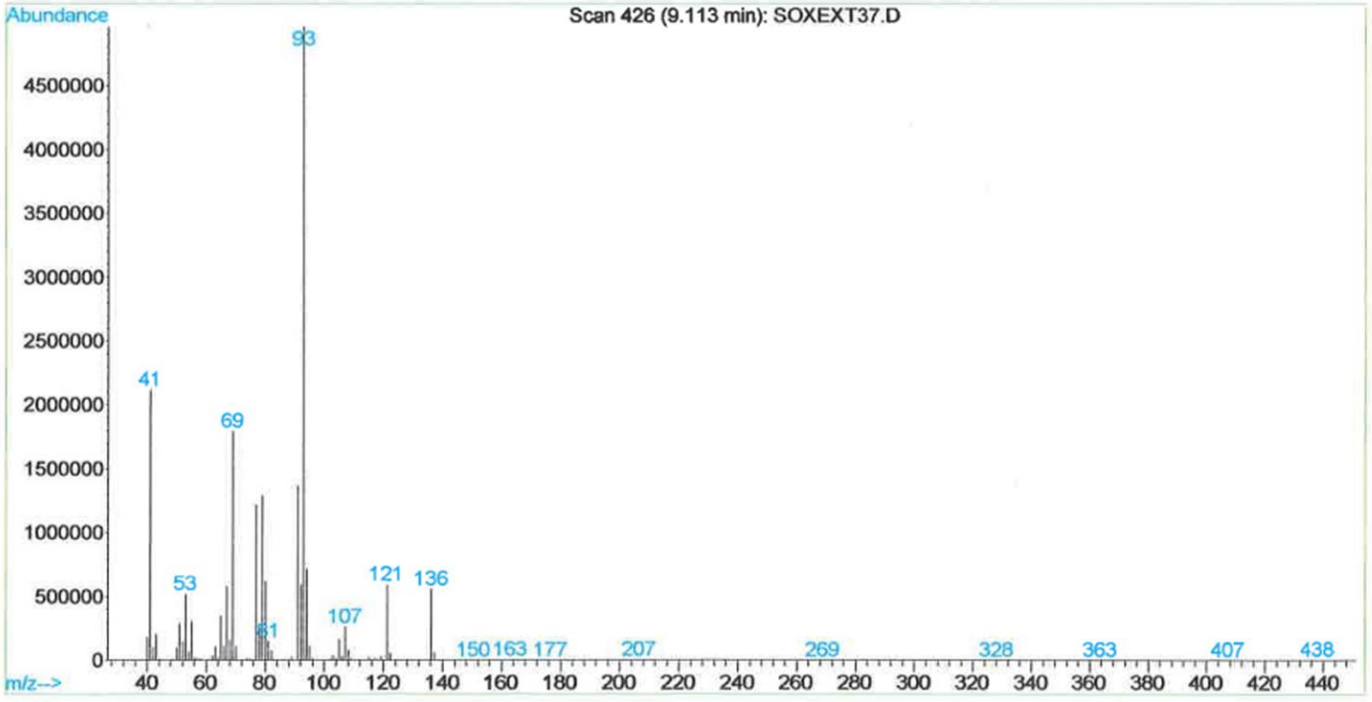


Figure A4. Chromatogram of Cis-Ocimene Standard

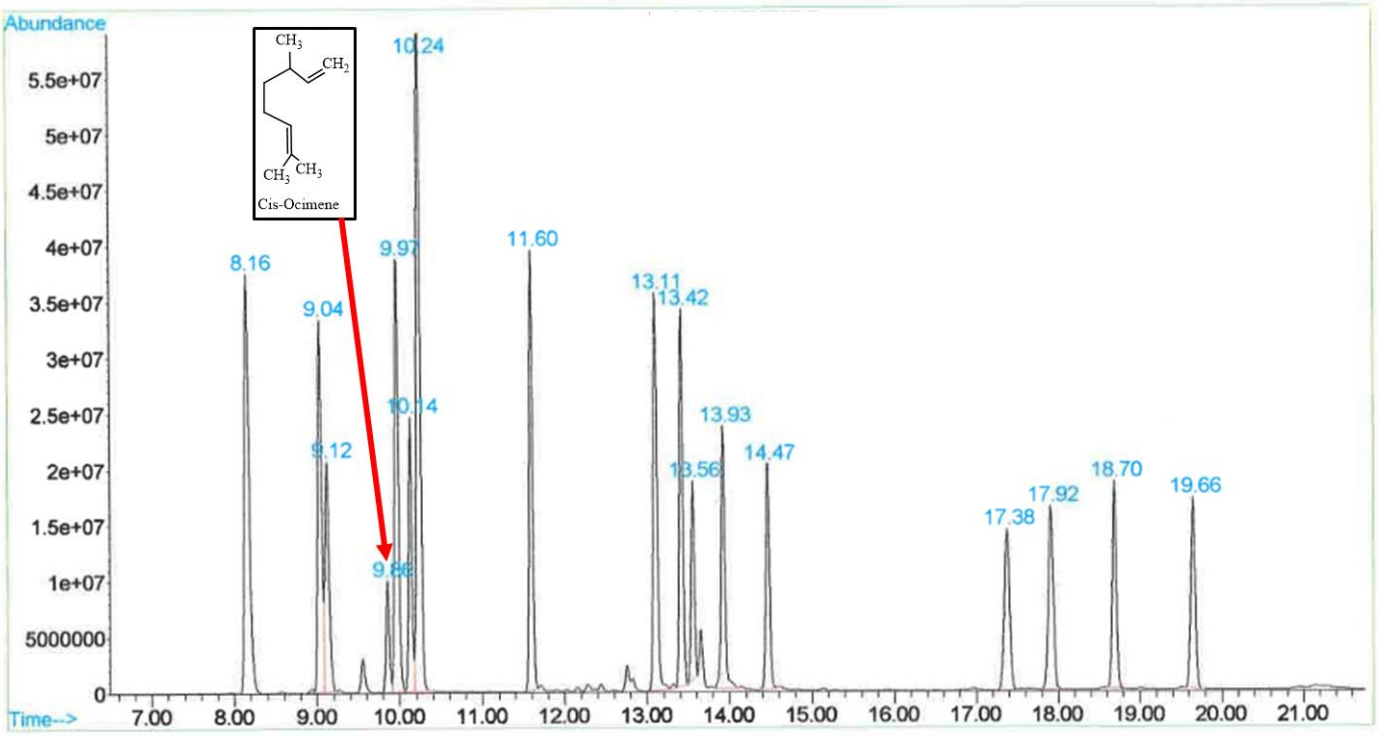


Figure A5. Chromatogram of Limonene Standard

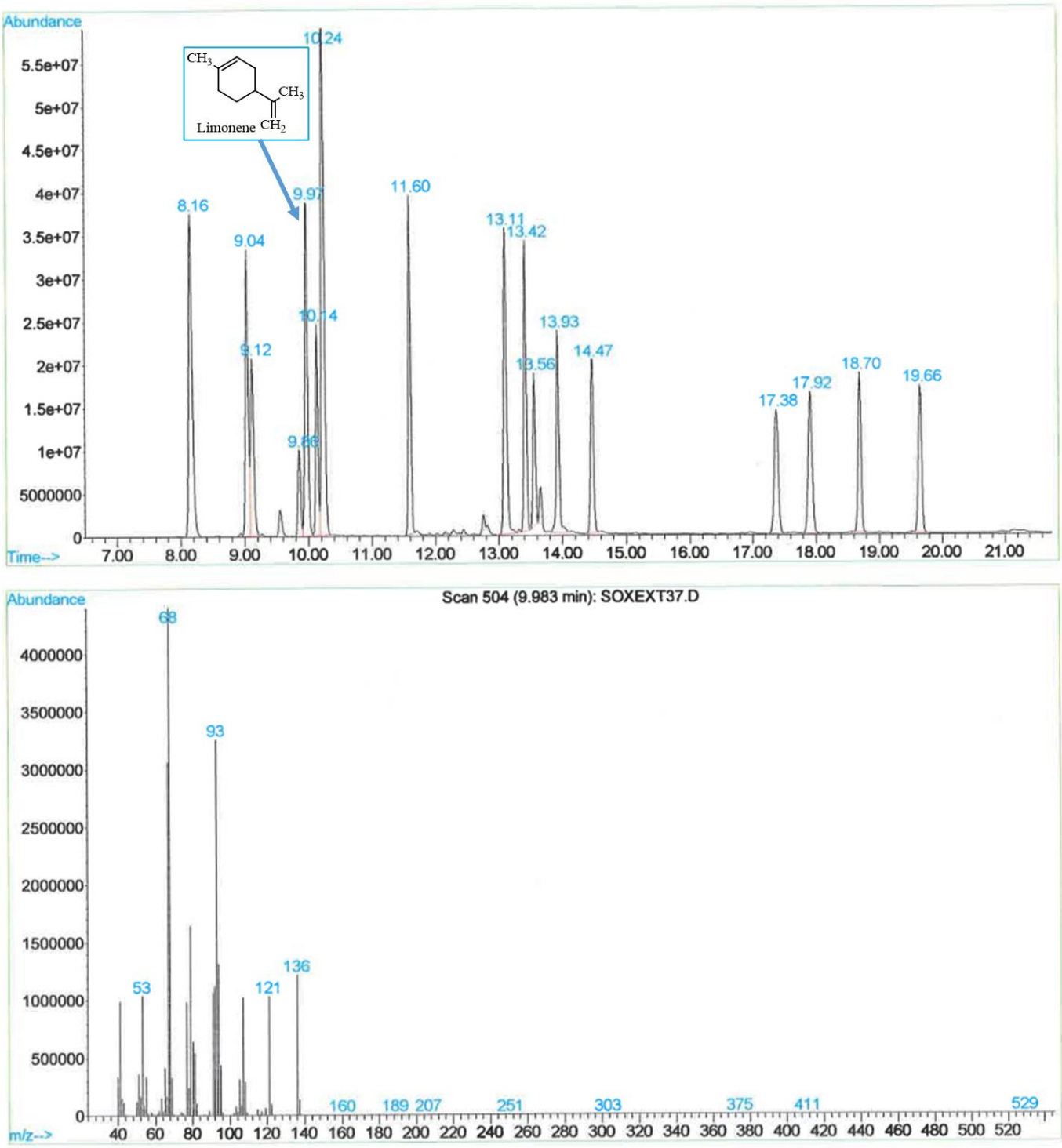


Figure A6. Chromatogram of Trans- Ocimene Standard

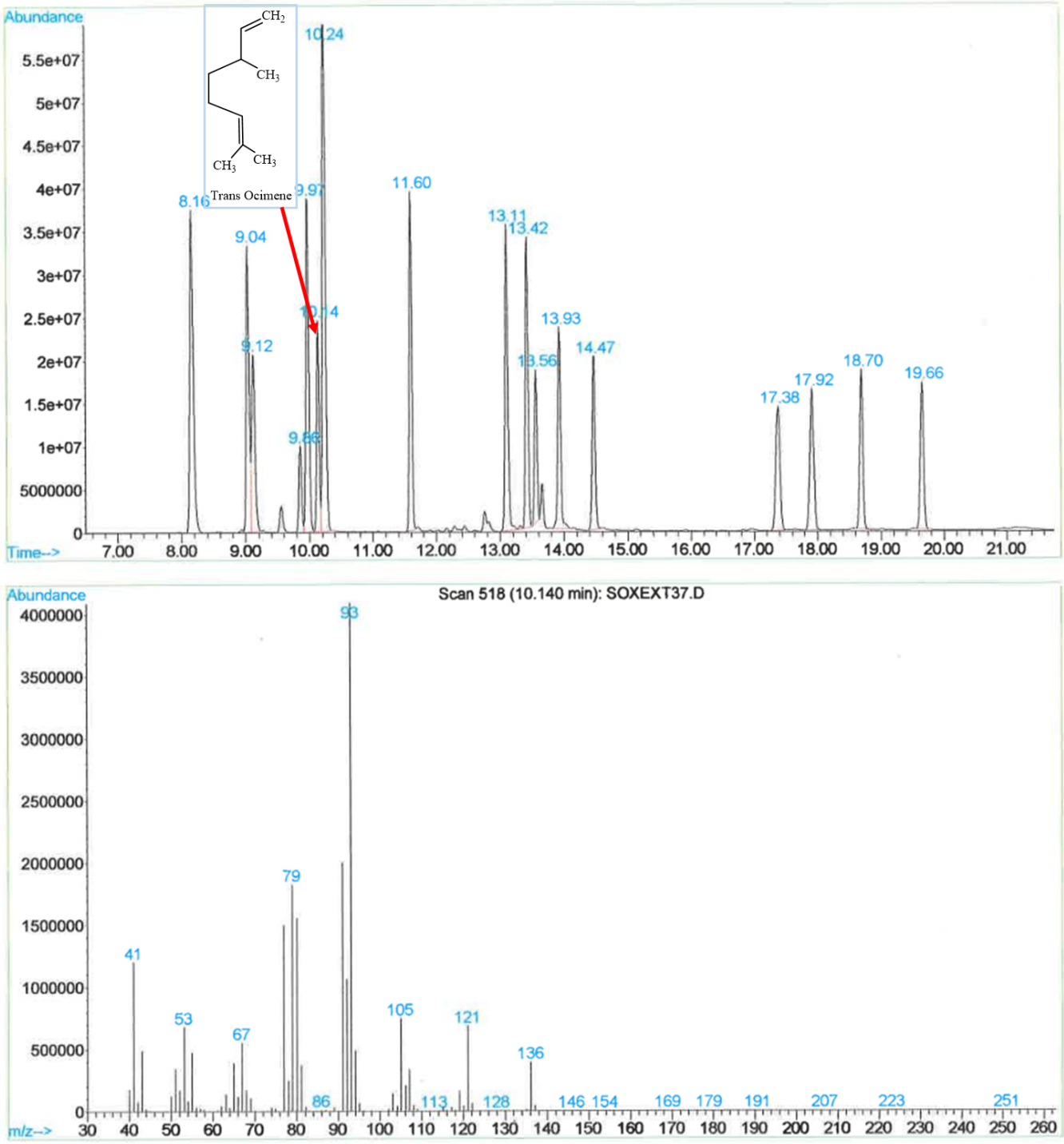


Figure A7. Chromatogram of Eucalyptol Standard

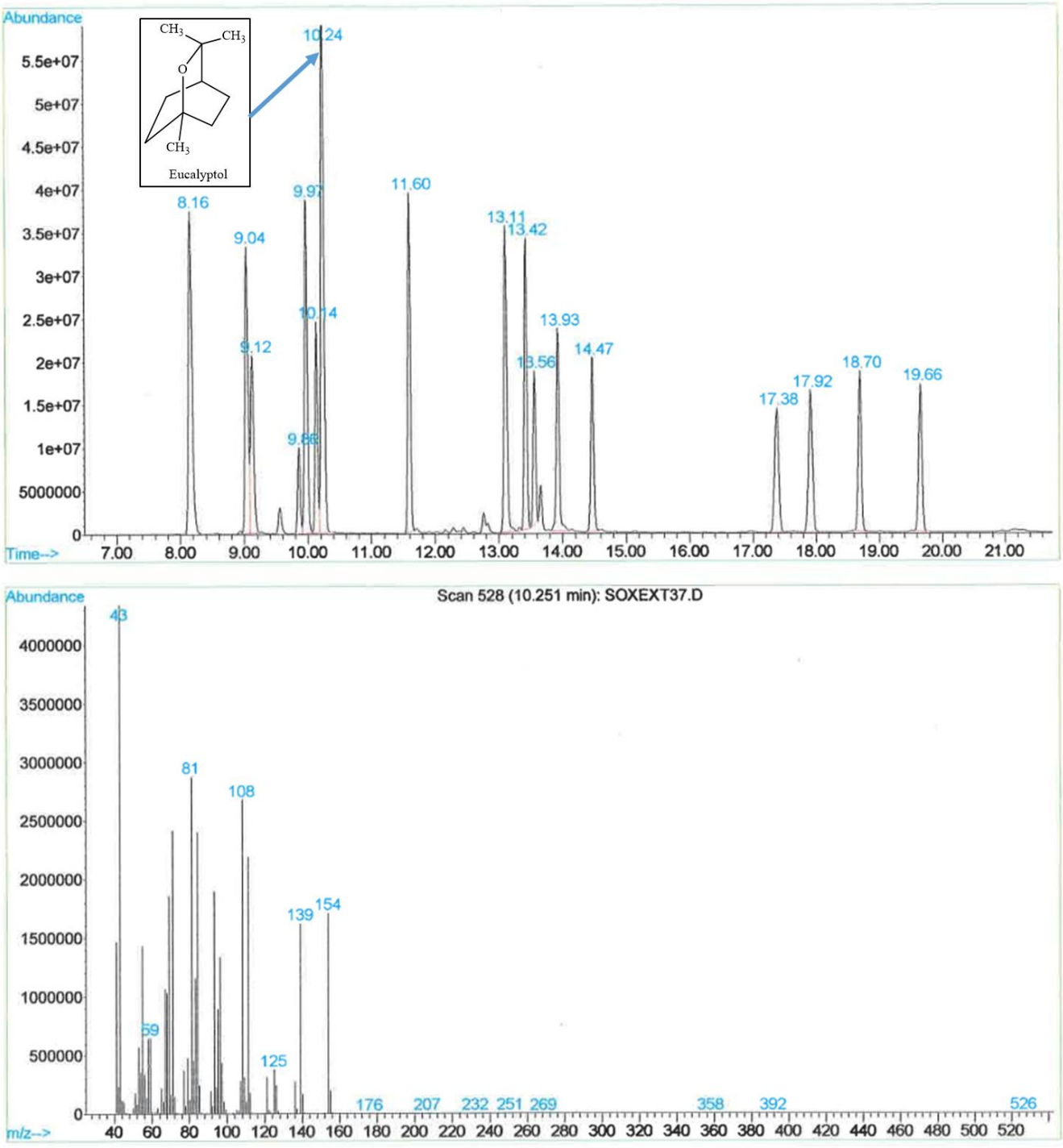


Figure A8. Chromatogram of Linalool Standard

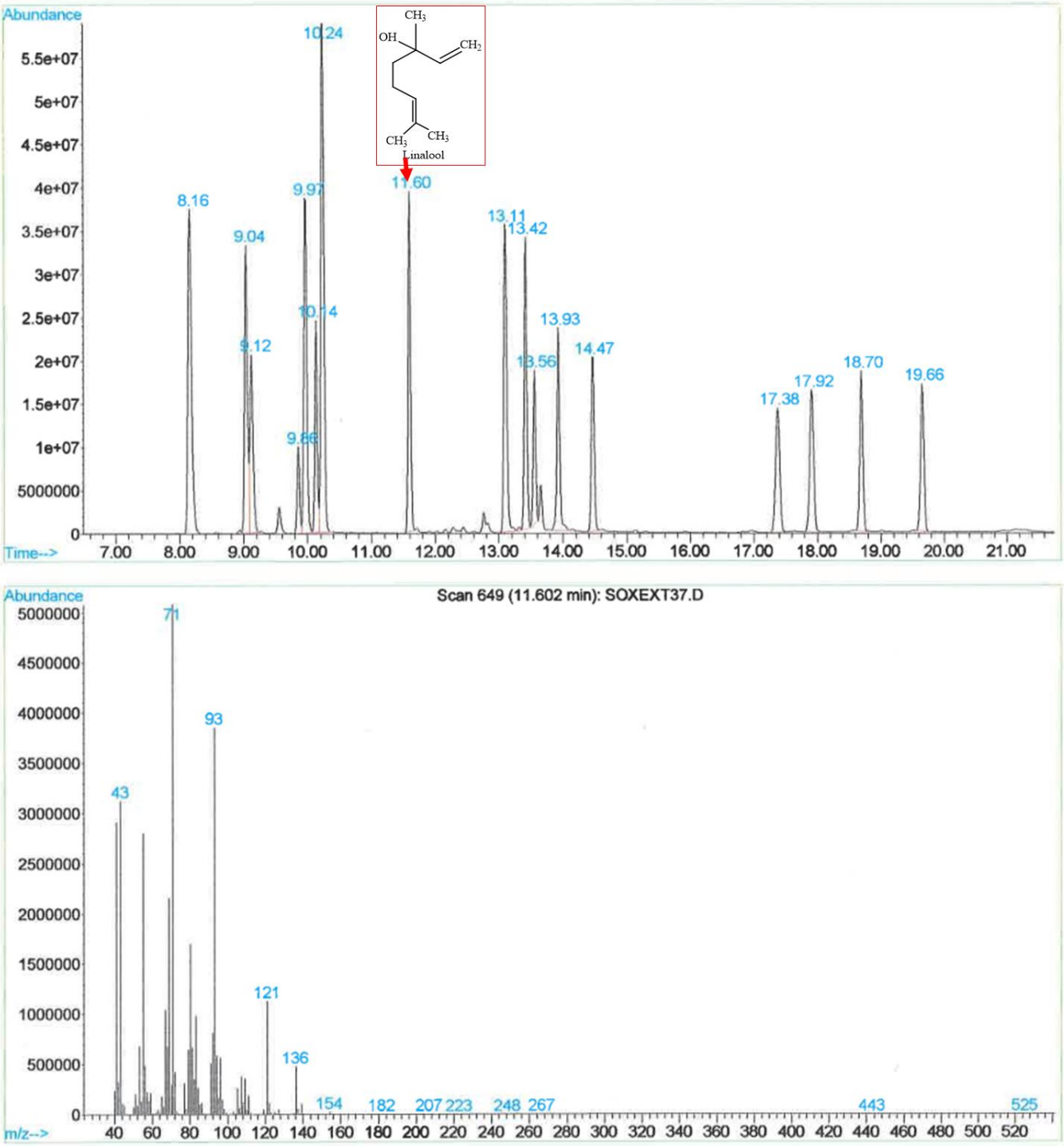
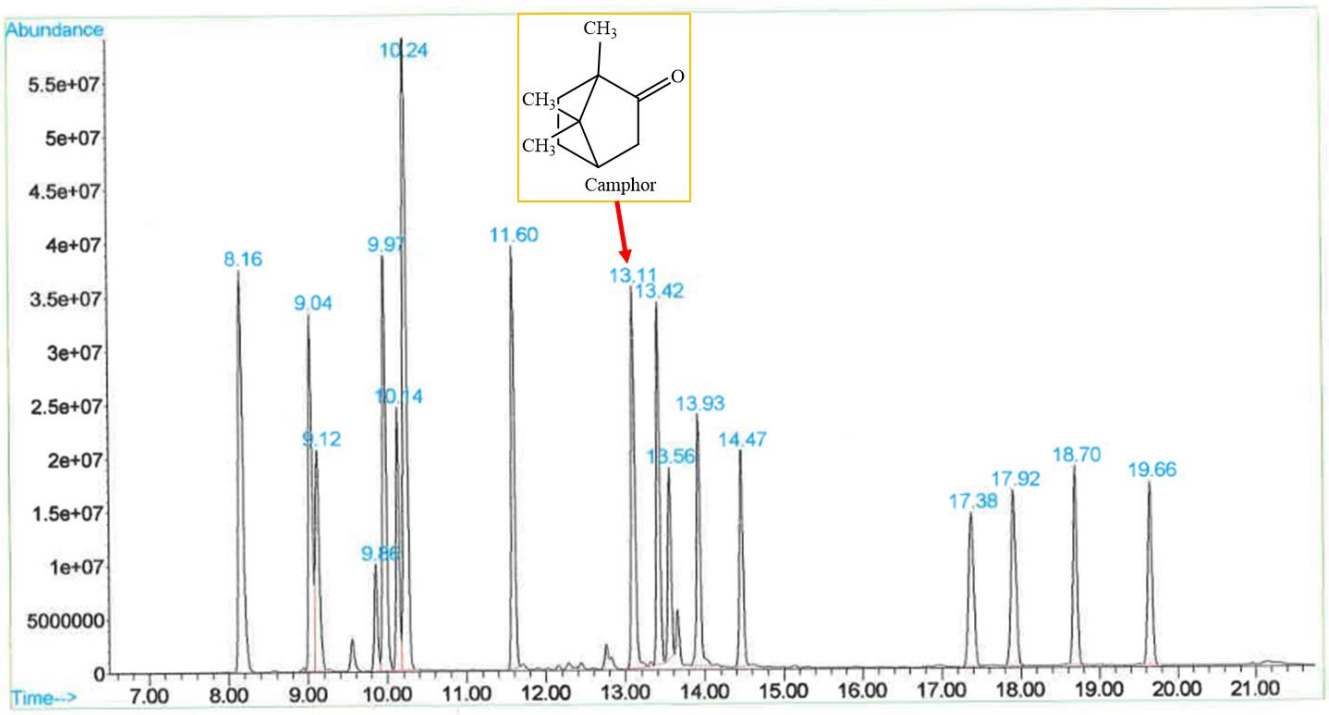


Figure A9. Chromatogram of Camphor Standard



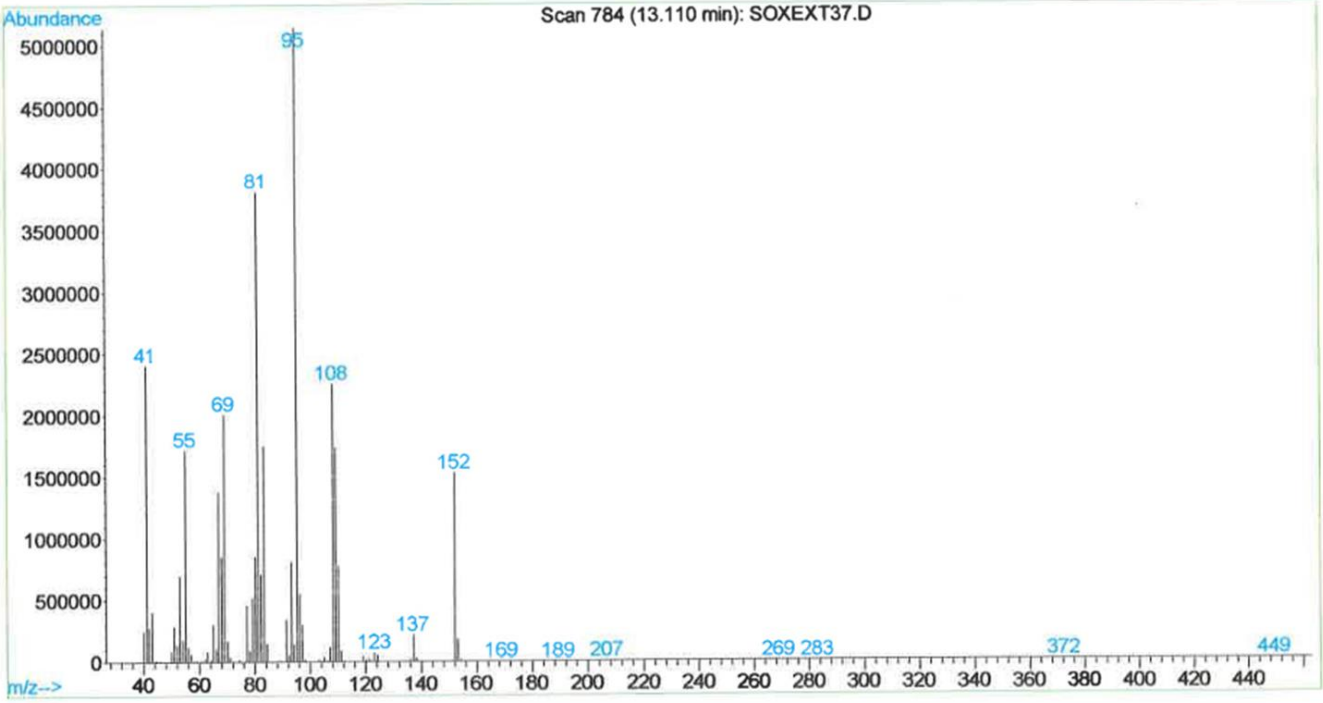
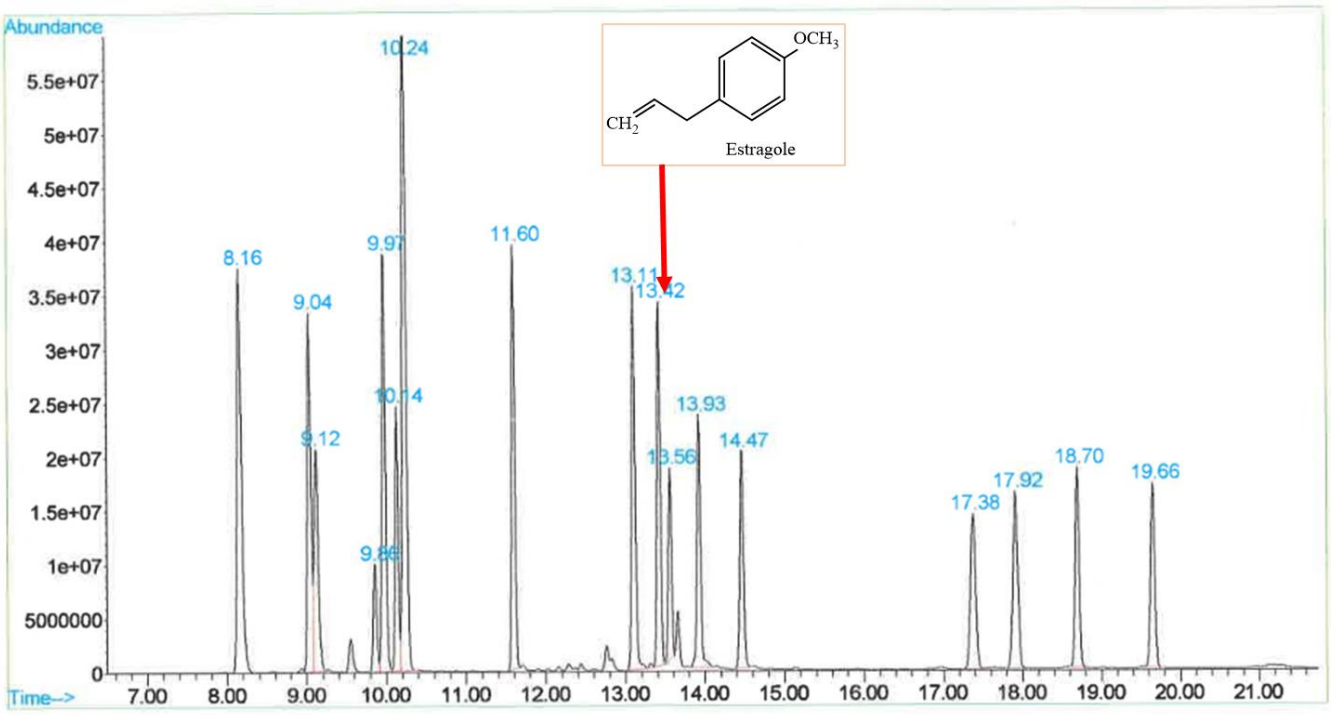


Figure A10. Chromatogram of Estragole Standard



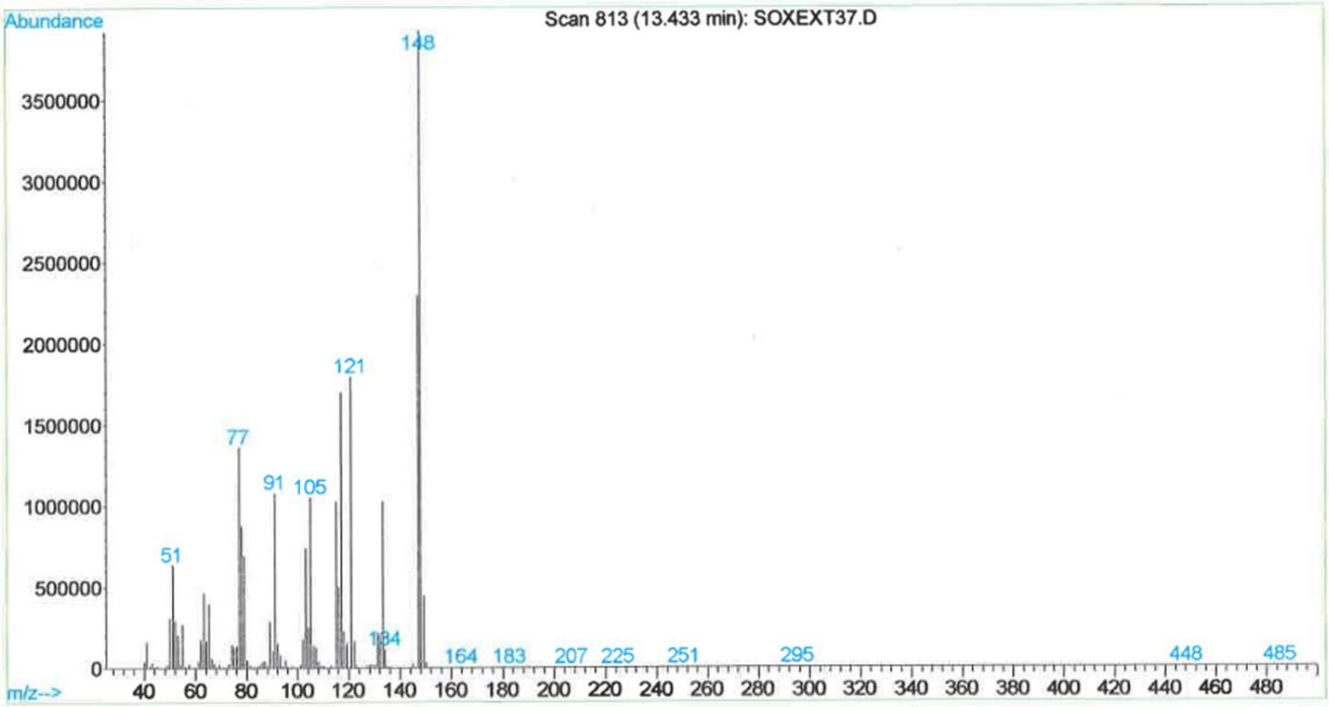


Figure A11. Chromatogram of Terpineol Standard

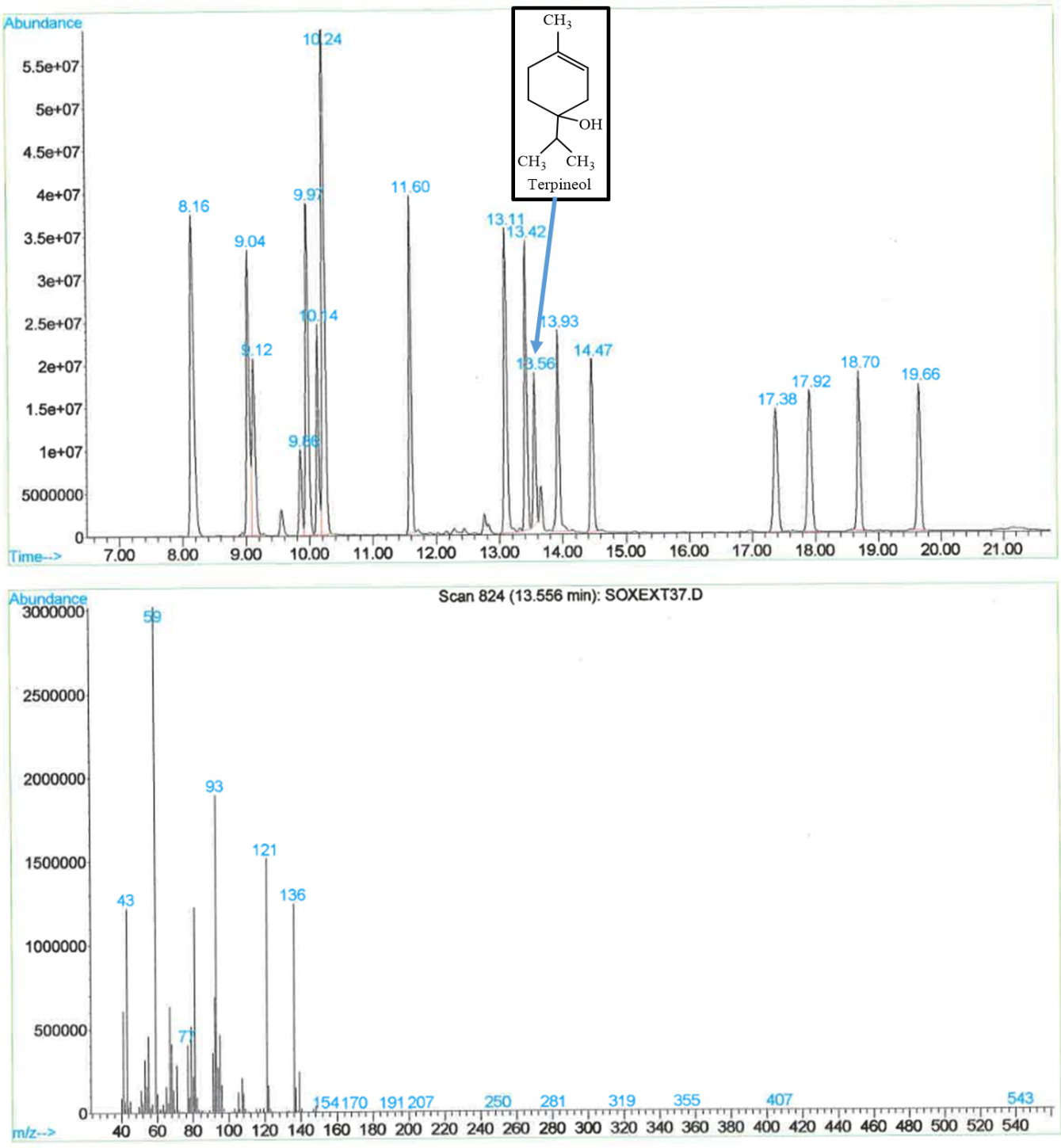


Figure A12. Chromatogram of Citronellol Standard

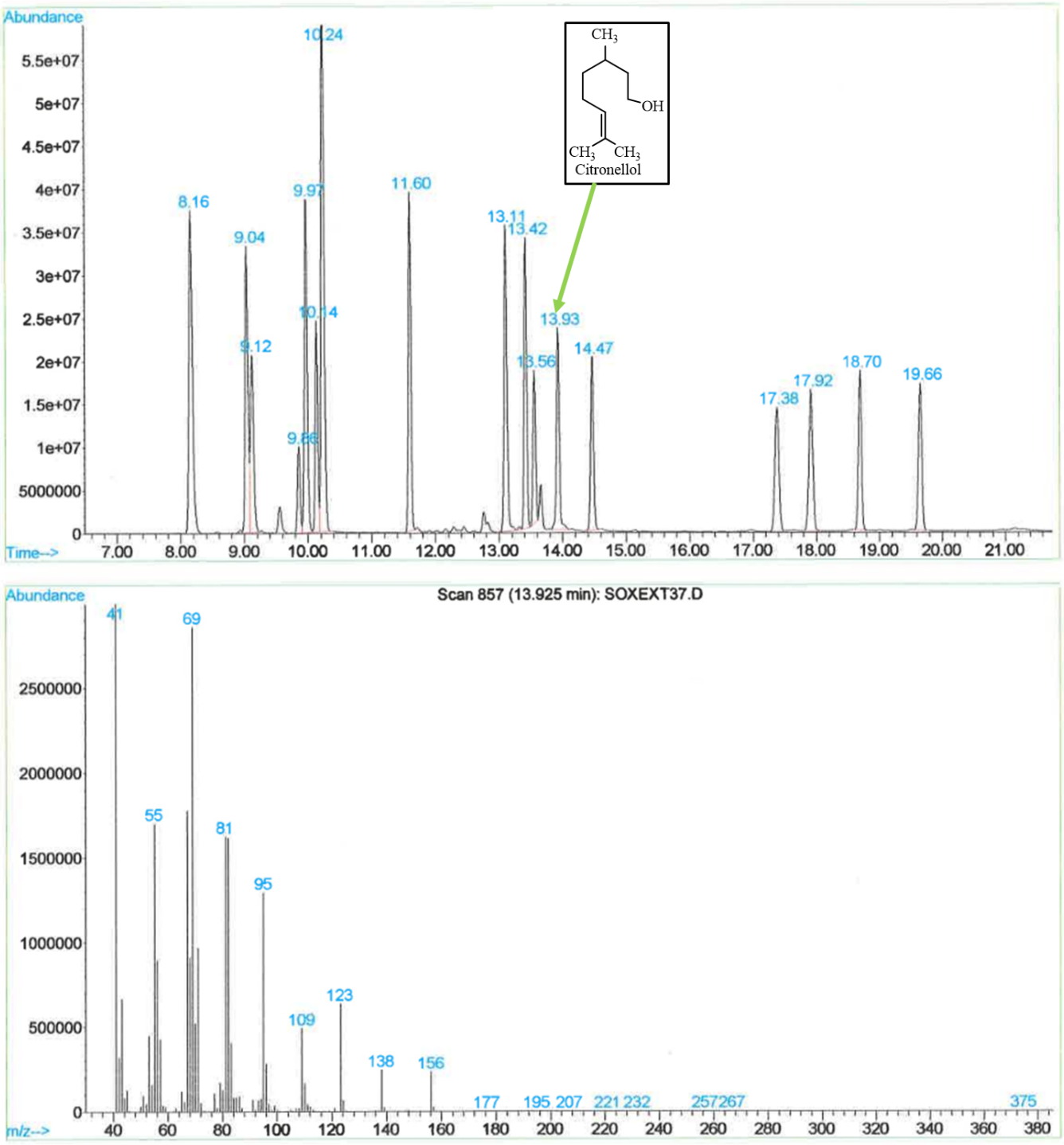
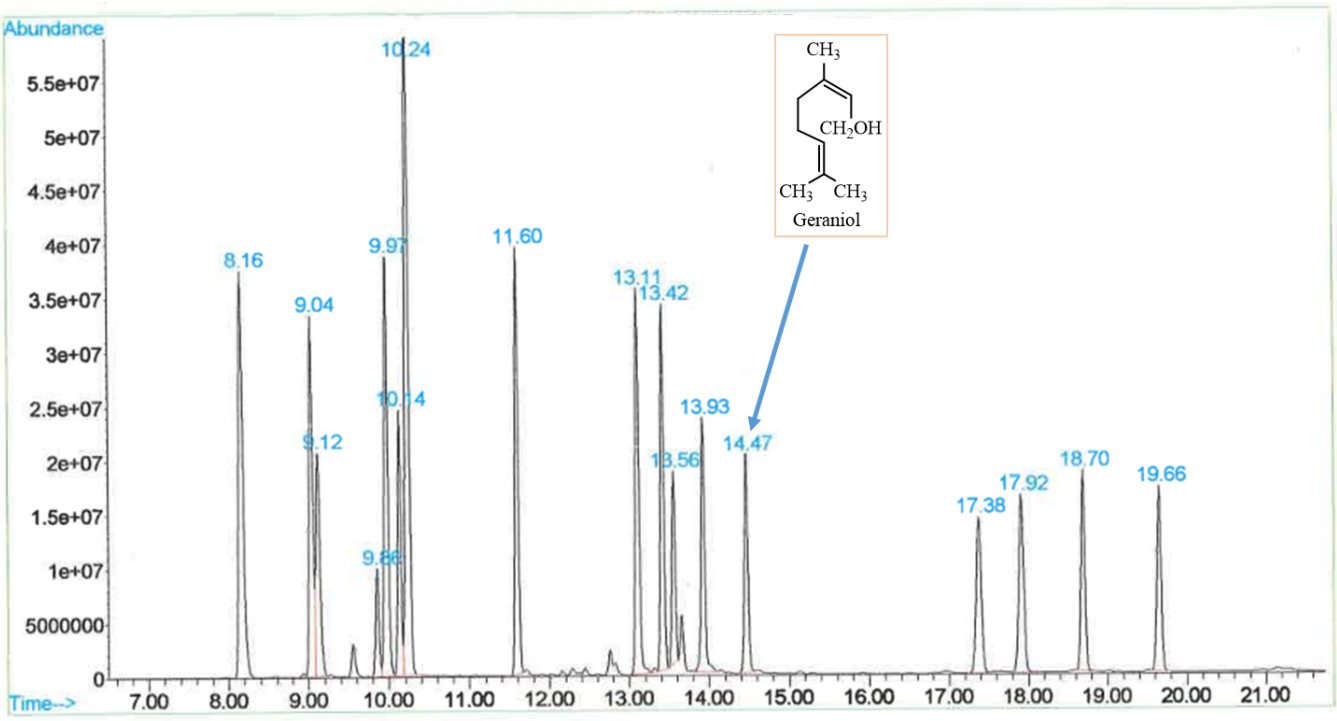


Figure A13. Chromatogram of Geraniol Standard



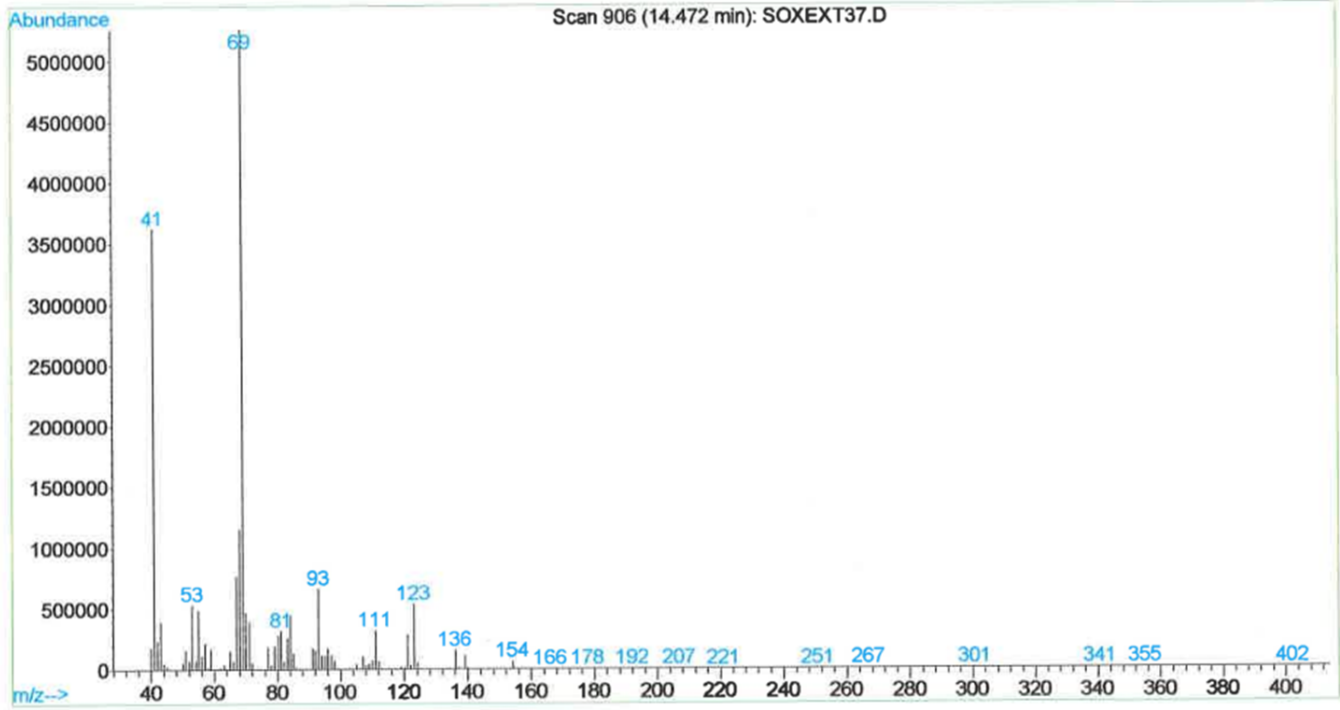


Figure A14. Chromatogram of Eugenol Standard

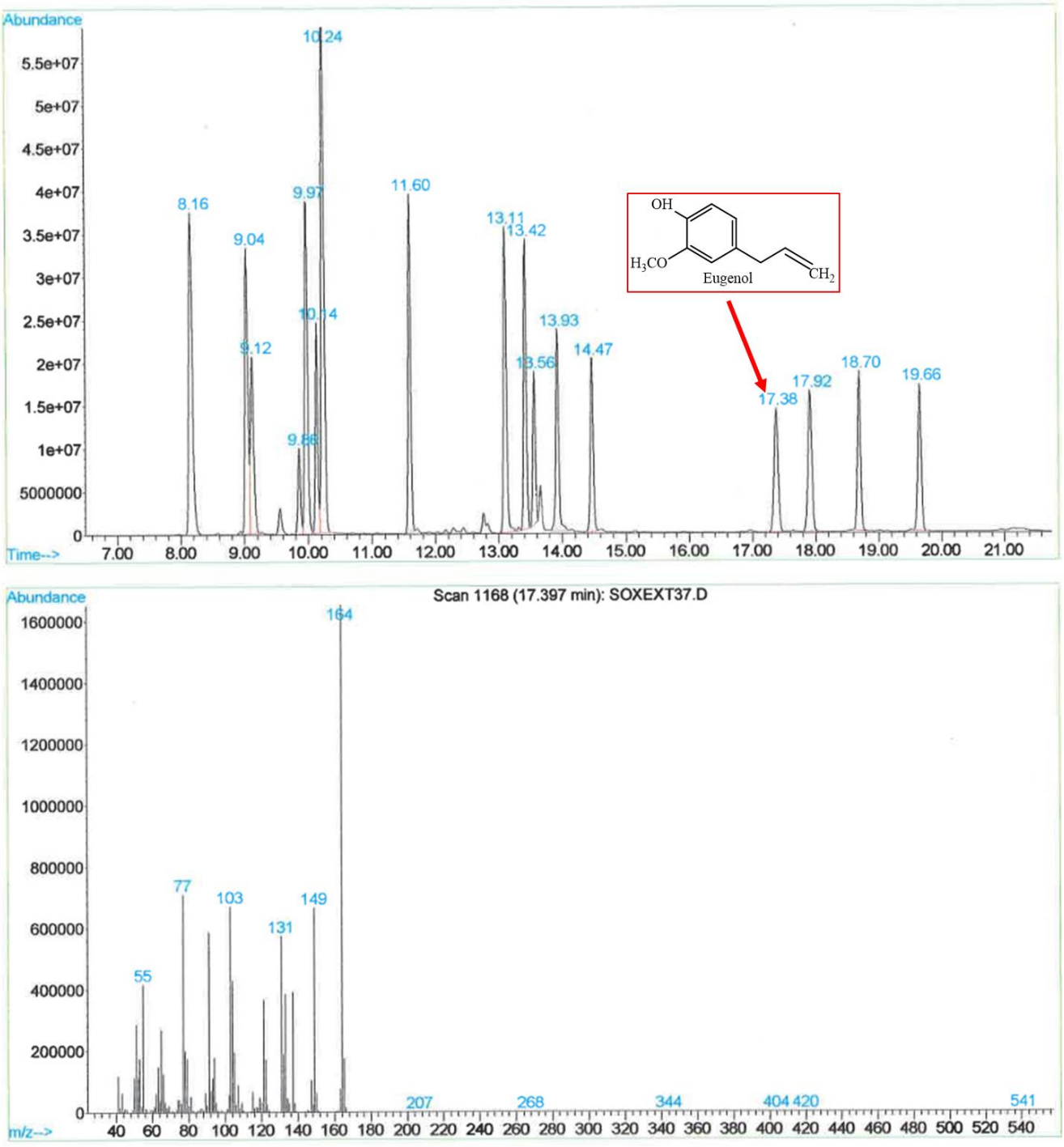


Figure A15. Chromatogram of Methyl Cinnamate Standard

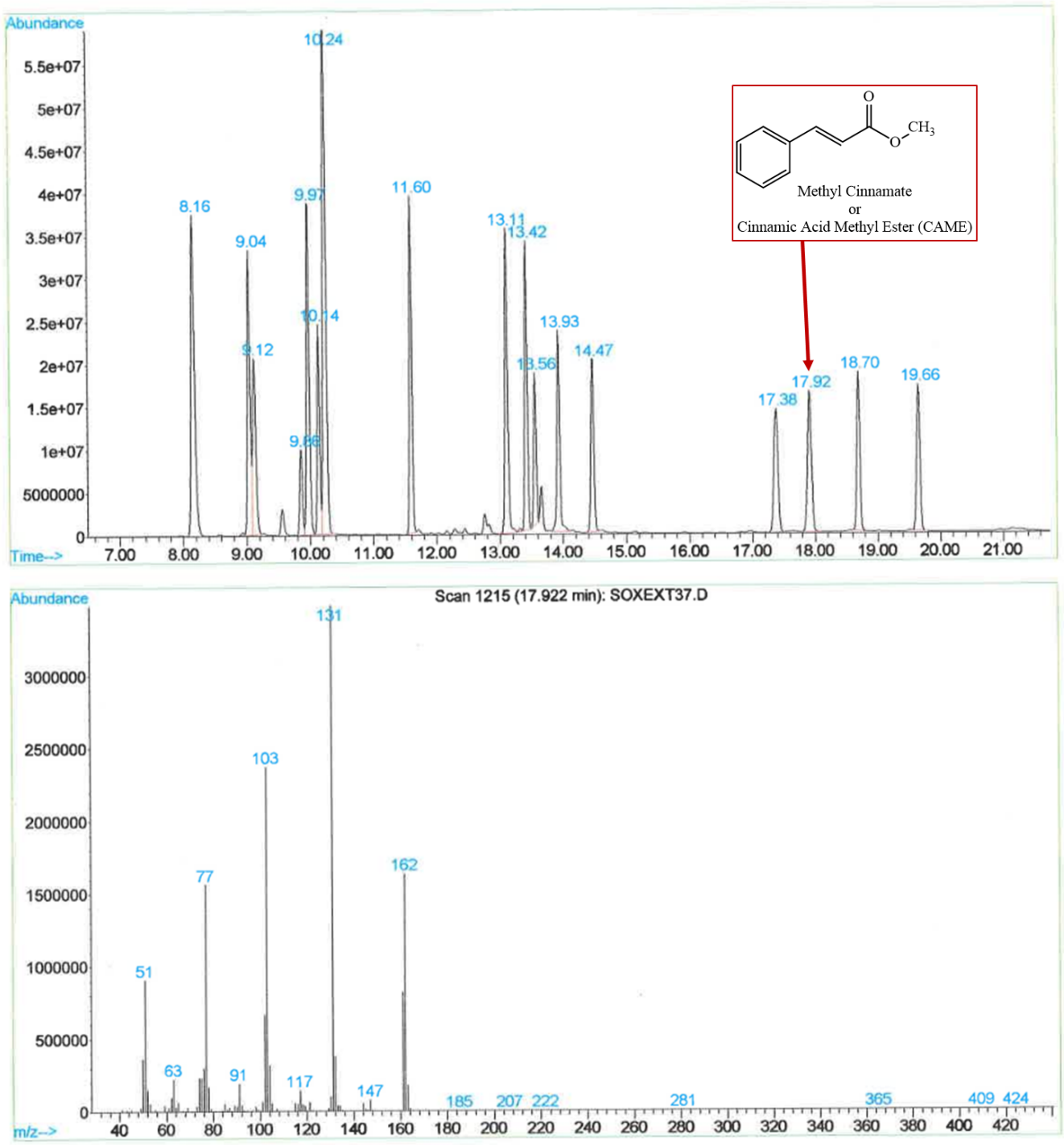
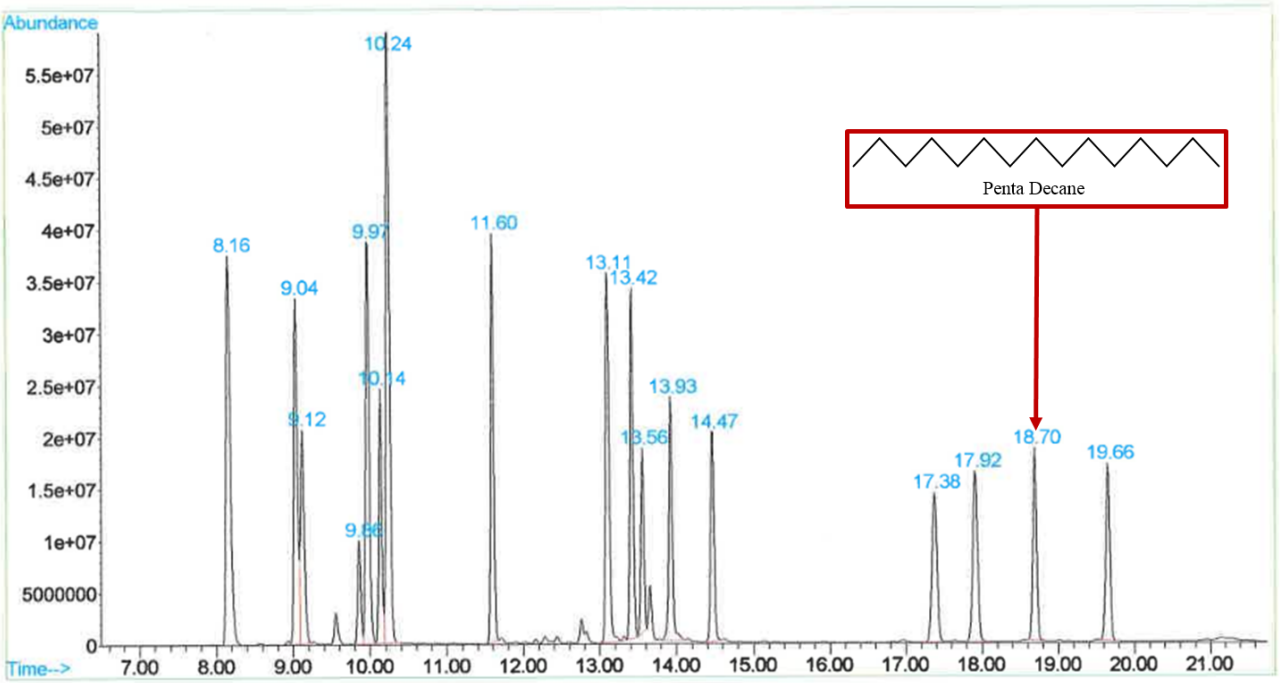


Figure A16. Chromatogram of Pentadecane Standard



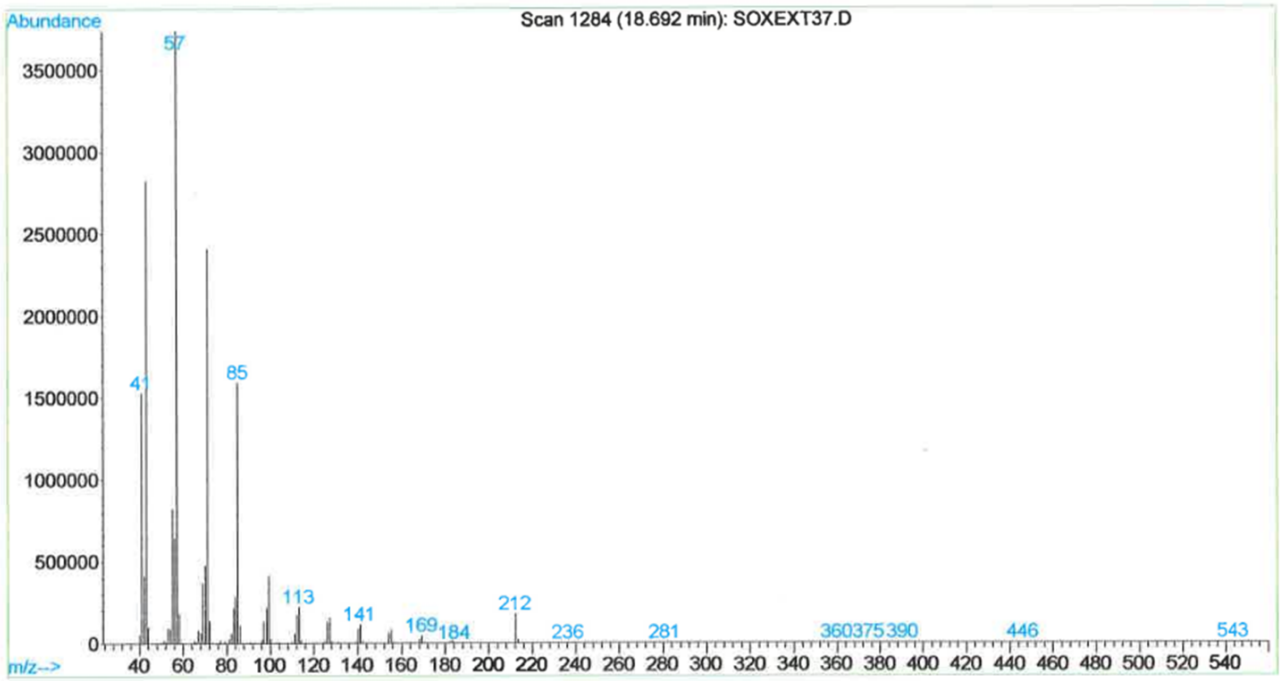
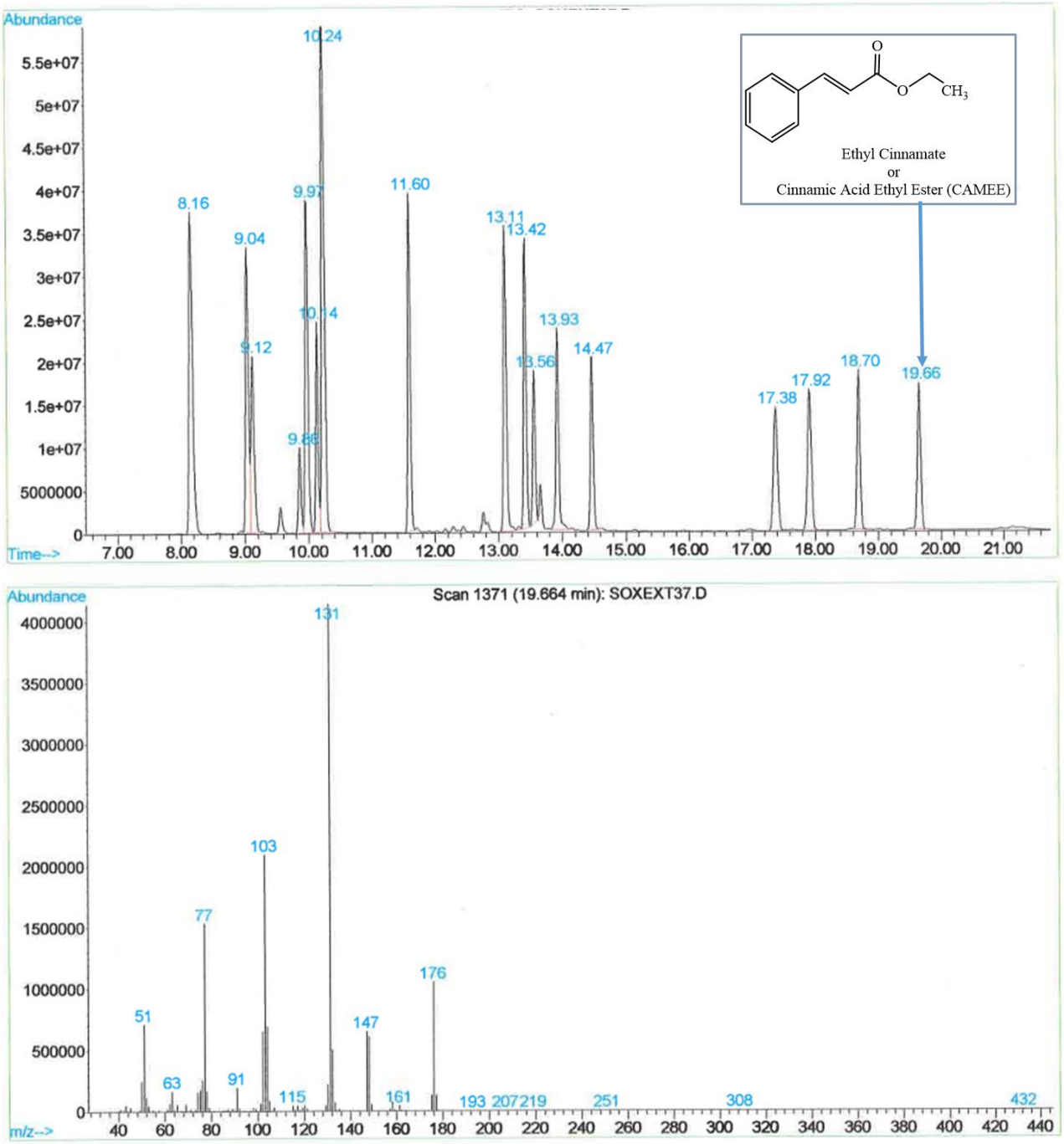


Figure A17. Chromatogram of Ethyl Cinnamate Standard



Appendix B

Table B1. Standard excel spreadsheet used to calculate the concentrations of the individual components found in basil extracts

Calculations for concentration standard stock solution of oils

		#	Name	V init	Density	Mass calc	Volume solut*
				µl		mg	ml
Initial stock standard mix sample							
Volume of each component, µl	100	1	a-Pinene	100	0.858	85.8	11.5
Total Volume stock in hexane, ml	11.5	2	Myrcene	100	0.791	79.1	11.5
		3	b-Pinene	100	0.859	85.9	11.5
		4	Limonene	100	0.841	84.1	11.5
Initial standard solut		5	Ocimene	100	0.818	81.8	11.5
Internal standard	PentaDecane	6	Eucalyptol	100	0.922	92.2	11.5
Mass taken, g	1	7	Linalool	100	0.870	87	11.5
Volume solvent hexane, ml	100	8	Camphor	100	0.990	99	11.5
Concentration, mg/ml	10	9	Estragol	100	0.965	96.5	11.5
		10	Terpinol	100	0.934	93.4	11.5
Make GC sample		11	Citronelol	100	0.855	85.5	11.5
Take stock, µl	600	12	Geraniol	100	0.879	87.9	11.5
Take Int stand sol, µl	600	13	Eugenol	100	1.067	106.7	11.5
		14	Me-Cin	100	1.090	109	11.5
Extracted solution to volume,ml	5	15	Pent decane				
		16	Et-Cin	100	1.050	105	11.5

* = 10 ml hexane + 1.5 ml total oil = 11.5 cc

** - IS solution is Pentadecane in hexanes, 10 mg /ml

Solution for analysis made in GC vilas by taking:

xx µl of Stock and xx µl of IS solution ** 600µl

Concentr mg/ml	Name	Concentr mg/ml	Conc PentDec mg/ml	Ratio Cx/ C int stand
7.5	a-Pinene	3.73	5.00	0.7461
6.9	Myrcene	3.44	5.00	0.6878
7.5	b-Pinene	3.73	5.00	0.7470
7.3	Limonene	3.66	5.00	0.7313
7.1	Ocimene	3.56	5.00	0.7113
8.0	Eucalyptol	4.01	5.00	0.8017
7.6	Linalool	3.78	5.00	0.7565
8.6	Camphor	4.30	5.00	0.8609
8.4	Estragol	4.20	5.00	0.8391
8.1	Terpinol	4.06	5.00	0.8122
7.4	Citronelol	3.72	5.00	0.7435
7.6	Geraniol	3.82	5.00	0.7643
9.3	Eugenol	4.64	5.00	0.9278
9.5	Me-Cin	4.74	5.00	0.9478
	PentaDec	5.00	5.00	1.0000
9.1	Et-Cin	4.57	5.00	0.9130

Area report standart mix. Taken from CF 109

#	Comp	S (comp)	S (IS)	S x/S IS	RF = (Cx/Cis)/ (Sx/Sis)
	Taken from CF 109				
1	a-Pin	368.7433	447.4328	0.824	1.76683889
2	Myr	300.04	447.4328	0.671	1.77526552
3	b-Pin	275.9374	447.4328	0.617	2.73860538
4	Lim	365.479	447.4328	0.817	1.48581591
5	Ocim	330.44	447.4328	0.739	1.62034786
6	Eucalyptol	215.02	447.4328	0.481	2.05009
7	Lin	386.4582	447.4328	0.864	1.49501912
8	Cam	355.3666	447.4328	0.794	1.3459137
9	Est	463.847	447.4328	1.037	1.3516744
10	Terpinol	275.3046	447.4328	0.615	2.63106472
11	Cit	366.8368	447.4328	0.820	1.44654415
12	Ger	440.6678	447.4328	0.985	1.35407669
13	Eug	486.5762	447.4328	1.087	1.51162566
14	CA_me	340.2438	447.4328	0.760	1.52306346
15	PentaDec	447.4328	447.4328	1.000	0.99995665
16	CA_ee	454.7932	447.4328	1.016	1.31495883

Table B2. Concentration of the basil oil extracts derived from various sections of the plant after plasma treatment by the standard protocol.

Absolute Concentrations (mg) in the 5 mL volumetric flask in mg quantity

1. Section DI-DL is wet leeves (10g) hexane extraction
2. Section DM-DP is wet stems (10g), Hexane extraction
3. Section DQ-DT is wet flowers (5g) , Hexane extract
4. Section DY-EB is wet mix (10g), Ethanol extract.

Symbol M stands for mix. Symbol A=control,
Symbol B=seed treated, Symbol C=body treatment 1x week for 30sec, D=body treatment 2x per week (30 sec)

Compone	GAL	GBL	GCL	GDL	GAS	GBS	GCS	GDS	GAF	GBF	GCF	GDF	GAM	GBM	GCM	GDM	GAM-ET	GBM-ET	GCM-ET	GDM-ET
a-Pin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Myr	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
b-Pin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lim	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.135	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ocim	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Eucalyptol	0.522	0.232	0.791	0.797	0.000	0.000	0.000	0.000	0.575	0.586	0.542	0.633	0.940	0.313	0.390	0.665	0.804	0.348	1.028	1.089
Lin	0.445	0.224	0.391	1.726	0.174	0.000	0.000	0.000	9.893	4.714	5.655	8.829	3.523	1.724	2.021	2.402	2.516	1.693	5.723	5.773
Cam	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Est	5.354	1.971	4.130	7.897	0.265	0.000	0.200	0.323	5.566	2.430	1.096	2.609	3.047	4.091	4.443	3.500	5.633	2.690	7.552	7.106
Terpinol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cit	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ger	0.552	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Eug	0.322	0.099	0.342	0.000	0.000	0.000	0.000	0.000	0.434	0.375	1.043	0.274	0.545	0.000	0.299	1.474	0.324	0.000	0.950	0.733
CA_me	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.361	0.000	0.457	0.209	0.128	0.000	0.111	0.000	0.503	0.226	0.972	0.734
CA_ee	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.365	0.000	0.000	0.094	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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